AMERICAN FERN JOURNAL

Volume 96

Number 1

January–March 2006

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

The Ferns and Fern Allies of the Karst Forests of Bohol Island, Philippines
Julie F. Barcelona, Nemrod E. Dolotina, Gil S. Madroñero, William G. Granert and Diosdado D. Sopot **1**

Leaf Phenology of the Climbing Fern *Lygodium* venustum in a Semideciduous Lowland Forest on the Gulf of Mexico
Klaus Mehltreter **21**

***Megalastrum* (Dryopteridaceae – Pteridophyta) in Bolivia, with Descriptions of Six New Species**
Michael Kessler and Alan R. Smith **31**

The American Fern Society

Council for 2006

TOM RANKER, University Museum, Campus Box 265, University of Colorado, Boulder, CO 80309-0265.
President

DAVID S. CONANT, Dept. of Natural Sciences, Lyndon State College, Lyndonville, VT 05851.
President-Elect

W. CARL TAYLOR, 800 W. Wells St., Milwaukee Public Museum, Milwaukee, WI 53233-1478.
Secretary

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916-1110.
Treasurer

GEORGE YATSKIEVYCH, Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299.
Membership Secretary

JAMES D. MONTGOMERY, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.
Curator of Publications

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Journal Editor

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Memoir Editor

ROBIN HALLEY, 1418 Park Row, La Jolla, CA 92037-3710
DAVID SCHWARTZ, 9715 Christey Way, Bakersfield, CA 93312-5617
Bulletin Editors

American Fern Journal

EDITOR

R. JAMES HICKEY ..... Botany Department, Miami University, Oxford, OH 45056,
ph. (513) 529-6000, e-mail: hickeyrj@muohio.edu

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47405-6801
GARY K. GREER ..... Biology Dept., Grand Valley State University, Allendale, MI 49401
CHRISTOPHER H. HAUFLER ..... Dept. of Ecology and Evolutionary Biology, University of Kansas,
Lawrence, KS 66045-2106
ROBBIN C. MORAN ..... New York Botanical Garden, Bronx, NY 10458-5126
JAMES H. PECK ..... Dept. of Biology, University of Arkansas–Little Rock,
2801 S. University Ave., Little Rock, AR 72204

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at The American Fern Society, % Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299. Periodicals postage paid at St. Louis, MO, and additional entry.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.

Changes of address, dues, and applications for membership should be sent to the Membership Secretary.

General inquiries concerning ferns should be addressed to the Secretary.

Back volumes are avalible for most years as printed issues or on microfiche. Please contact the Back Issues Curator for prices and availability.

Subscriptions:

Society Membership – USA, Canada, Mexico (includes Journal and Fiddlehead Forum)	$25
Society Membership – All other countries (includes Journal and Fiddlehead Forum)	$32
Society Life Membership – $300 (add $140 mailing surcharge for outside USA, Canada, Mexico)	
Regular Membership – USA, Canada, Mexico (includes Fiddlehead Forum)	$12
Regular Membership – All other countries (includes Fiddlehead Forum)	$19
Institutional Membership – $35 to USA, Canada, Mexico; $45 elsewhere (-$2 agency fee)	

POSTMASTER: Send address changes to American Fern Journal, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

American Fern Journal 96(1):1–20 (2006)

The Ferns and Fern Allies of the Karst Forests of Bohol Island, Philippines

JULIE F. BARCELONA
Philippine National Herbarium (PNH), Botany Division, National Museum of the Philippines, P. Burgos St., Manila, P.O. Box 2659, Philippines
NEMROD E. DOLOTINA, GIL S. MADROÑERO,
WILLIAM G. GRANERT and DIOSDADO D. SOPOT
Soil and Water Conservation Foundation of the Philippines, Inc. (SWCF),
169-G Ma. Christina Extension, Capitol Site, Cebu City,
6000 with field offices in Bilar and Sierra Bullones, Bohol, Philippines

ABSTRACT.—We provide the first and only comprehensive list of the ferns and fern allies of Bohol island, Philippines. This compendium is based on collections made from those of Cuming in 1841 to our recent survey of the karst forests of the Rajah Sikatuna Protected Landscape (RSPL) and vicinity during November, 2003 to February 2004. A total of 169 species in 63 genera and 27 families are reported here for the island, of which 91 species are new additions. Twenty-one species are Philippine endemics, five of which are previously known only from types or from very few collections. They are *Ctenitis boholensis*, *C. humilis*, *Pteris whitfordii*, *Antrophyum williamsii*, and *Lomagramma merrillii*. Bohol is the type locality of five fern names, namely, *Ctenitis humilis*, *C. boholensis*, *Cyclosorus glaber* and *Thelypteris sevillana* (= *Pneumatopteris glabra*), *Cyclosorus boholensis* Copel. (= *Sphaerostephanos acrostichoides*), and *Diplazium petiolare*. Two species in the genera *Oleandra* and *Ctenitis* and several specimens of *Selaginella* remain undetermined. Nineteen previously reported species have not been recollected or in some cases, their taxonomic identities are doubtful. Prior to this study, only one fern specimen from Bohol was accessioned at the Philippine National Herbarium (PNH). All historical collections are currently deposited in herbaria in the U.S.A. and Europe. Boholanos have successfully maintained the integrity of the wild populations of ferns, especially those species that are heavily collected in other parts of the Philippines for their ornamental value.

Bohol is one of the least biologically explored islands in the Philippines. In the last century and a half, botanical explorations in Bohol have been few compared to other islands in the country (Amoroso *et al.*, 1995; Barcelona, 2002, 2003a and b, 2004; Copeland 1908, 1910; Hatusima, 1966; Iwatsuki and Price, 1977; Merrill, 1908). Historical collections include those of Cuming (in 1841), McGregor (in 1906 and 1910), Bartsch (Tagbilaran, in 1908), Ramos (Bilar, Dimiao, Sevilla, and Valencia, in 1923), Konyo and Edaño (in 1957), and Co (Dagohoy, Danao, Inabanga and Pilar, in 1995). All combined, these explorations have resulted in a little more than a hundred pteridophyte collection numbers, of which Ramos contributed more than 80 percent representing 75 species. Nearly all of these historical collections are now deposited in herbaria in the United States and Europe. Whereas Co's collections are deposited at PUH, University of the Philippines-Diliman, only one specimen from Bohol was accessioned at the Philippine National Herbarium (PNH) prior to the present study.

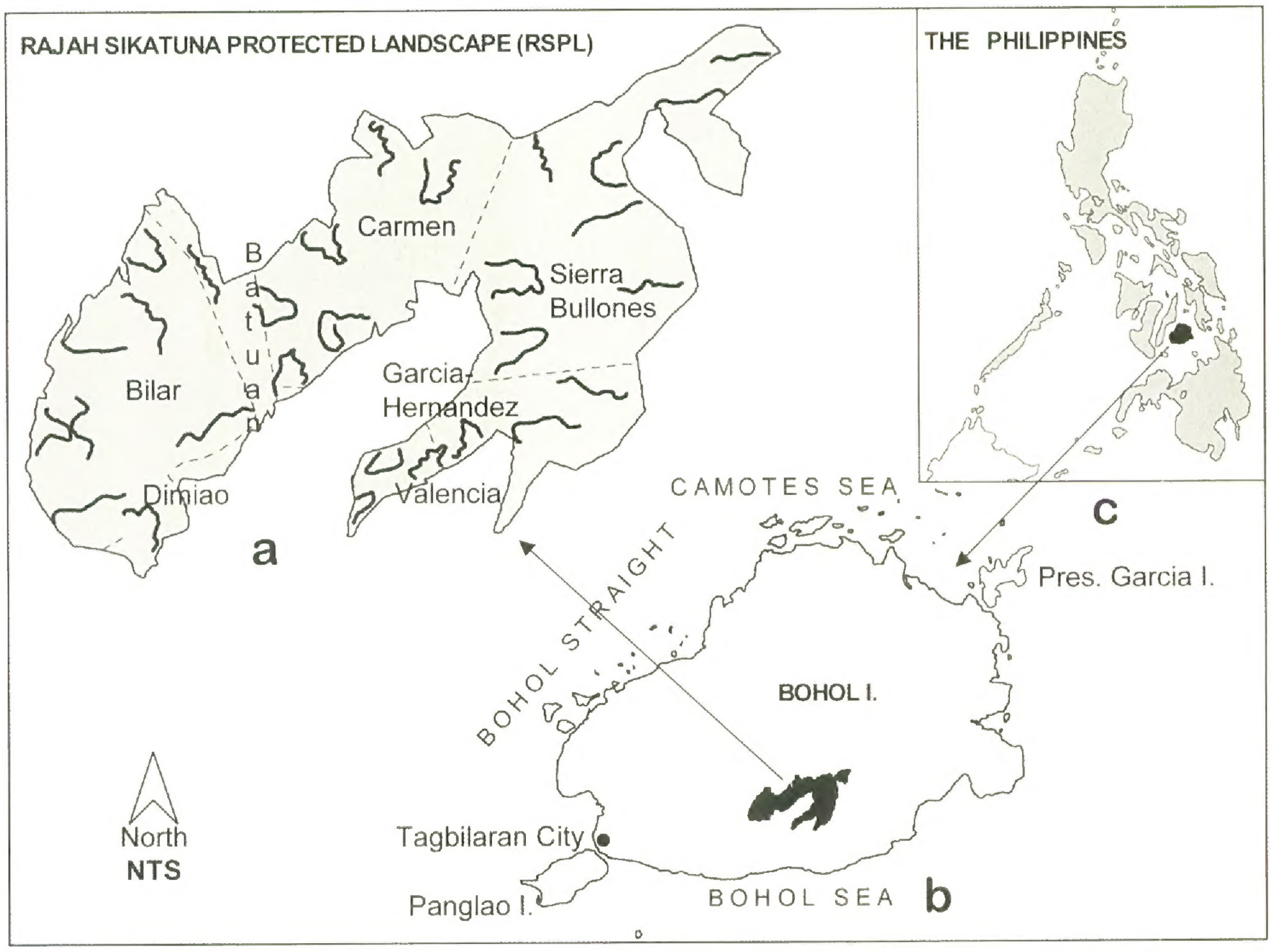


FIG. 1. Map of Rajah Sikatuna Protected Landscape (RSPL), Bohol Island, Philippines showing the Biodiversity Monitoring Trails (BMS) sampled. Map modified by the first author from the University of San Carlos/GIS-WRC Center and SWCF, 2001.

The Rajah Sikatuna Protected Landscape (RSPL) is one of the many protected areas so far established on Bohol. It encompasses forested karst topography characterized by dogtoothed terrain with many caves and sinkholes. The forest canopy is multi-layered with trees reaching 20m tall. The canopy includes members of the Dipterocarpaceae, Moraceae (*Ficus* spp.), and Meliaceae, among others. Some regions of the preserve have been reforested with *Gmelina arborea* and *Swietenia macrophylla*. The numerous canopy vines and lianas include the scrambling bamboos and species of *Strongylodon* and *Tetrastigma*. Some species of the latter genus are hosts to parasitic *Rafflesia*. Ferns and fern allies are dominant components of the understory and the epiphytic flora, especially in high-moisture areas. Unique species associations are evident in different microhabitats and on different substrates.

MATERIAL AND METHODS

During Nov 2003 and from Jan to Feb 2004, a reconnoissance survey and photo-documentation of RSPL (Fig. 1) was conducted. Following these activities, ferns and fern allies were collected from the limestone (karst)

TABLE 1. Survey localities for ferns and fern allies at Rajah Sikatuna Protected Landscape (RSPL) and vicinity, Bohol Island Province.

Municipality (= Town)	Barangay (= Barrio)	Elevation (m)
Bilar	Bugang Sur	330–365
	Cansumbol	300–370
	Riverside	330–560
	Yanaya	330–560
Garcia-Hernandez	Datag	600–703
	Cambuyo	600–703
Guindulman	Biabas	560–585
Sierra Bullones	Bugsoc	390–600
	Lataban	620–720
	Nan-od	530–623
Valencia	Anonang	445–525
	Botong	575–600
	Canlambong	390–465
	La Victoria	480–600
	Lantang	470–520
	Marawis	390–465
	Maubo	570–600
	Omjon	510–546

forests and vouchers deposited in PNH. Collections and surveys were conducted at the localities listed in Table 1.

Although these 18 localities represent only 10% or less of the total land area of RSPL and almost a negligible portion of the island of Bohol, they substantially represent the unique microhabitats within the karst landscape that characterize much of the island. Collections within RSPL were made in forest interiors within close proximity of the pre-established Biodiversity Monitoring System (BMS) trails.

RESULTS

A total of 169 species in 63 genera and 27 families of ferns and fern allies have been initially identified to occur in Bohol (Table 2). Twenty-one of these species are Philippine endemics and two are local endemics. Our recent survey has added 91 new species records for Bohol since botanical explorations in this island started in 1841. We also recollected rare local endemics that have been known either only from the types or from very few herbarium collections. One such rarity is *Ctenitis humilis* Holtt., which was first collected by Ramos in Bohol (without exact locality) in 1923 and later (1935) was also found in Mindoro by Bartlett. The identity of *C. humilis* as distinct from *C. boholensis* has been questioned: "... *C. humilis* maybe a dwarf habitat-form of *C. boholensis*; further collections are needed ..." (Holttum, 1991, p. 31). *Ctenitis boholensis* Holtt., an apparent Bohol island endemic, is known only from the type (*Ramos BS42983*, K and UC) and two other

TABLE 2. Ferns and Fern Allies of Bohol Island, Philippines (1841–2004). (* - new record for Bohol; † - not recollected). (1 - Bilar; 2 - Dimiao; 3 - Garcia-Hernandez; 4 - Guindulman; 5 - Sevilla; 6 - Sierra Bullones; 7 - Tagbilaran; 8 - Valencia) N.B.: *Barcelona et al.* collections are at PNH.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
ADIANTACEAE			
Adiantum caudatum L.*	*Barcelona et al. 2633*	Epilithic.	Limestone walls in exposed areas. 445–525m. Infrequent. (8)
A. malesianum Ghatak	*Barcelona et al. 2410*	Epilithic.	Limestone walls in exposed areas. 330–560m. Infrequent. (1)
A. philippense L.*	*Barcelona et al. 2645*	Epilithic.	Karst forest interior. 445–525m. Seen only once. (8)
A. tenerum Sw.*	*Barcelona et al. 2425*	Terrestrial.	Karst forest margin. 330–560m. Locally common. (1)
Pityrogramma calomelanos (L.) Link*	not collected	Terrestrial.	Disturbed, exposed areas. Weedy. (1)
Taenitis cordata (Gaud.) Holtt.	*Barcelona et al. 2618*	Terrestrial.	Dry karst forest. (2; 8). 390–465m. Rare.
ASPLENIACEAE			
Asplenium affine Sw.	*Barcelona et al. 2500*	Epiphytic.	Karst forest in shaded areas. 543–586m. Frequent. (1; 6)
A. caudatum G. Forst.	*Barcelona et al. 2634*	Terrestrial.	Karst forest margins. 330–560m. Infrequent. (1; 8)
A. cymbifolium Christ*	*Barcelona et al. 2670*	Epiphytic.	Karst forest interior. 600–703m. Infrequent. (3)
A. epiphyticum Copel.	*Barcelona et al. 2580*	Terrestrial then twining on saplings.	Karst forest and margins. 330–560m. Frequent. (1; 8)
A. lobulatum Mett. *ex* Kuhn*	*Barcelona et al. 2461*	Terrestrial.	Karst forest, along trails. (330–560m). Frequent. (1)
A. musifolium Mett.*	*Barcelona et al. 2458*	Epiphytic.	Karst forest interior. Also cultivated. Frequent. 330–560m. (1; 8)
A. nidus L.*	not collected	Terrestrial, epiphytic, and epilithic.	Terrestrial, epilithic, and epiphytic. Karst forest interior. Infrequent.
A. pellucidum Lam.	*Barcelona et al. 2508*	Epiphytic.	Karst forest interior. 530–703m. Infrequent. (1; 3; 6)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
A. persicifolium J. Sm. *ex* Mett.	*Barcelona et al. 2512*	Terrestrial and twining on saplings.	Karst forest interior. 330–586m. Frequent. (1; 6; 8)
A. phyllitidis Don*	*Barcelona et al. 2386*	Epilithic or epiphytic.	Karst forest interior. 330–720m. Locally frequent. (1; 6; 8)
A. polyodon G. Forst.	*Barcelona et al. 2453*	Terrestrial, epilithic, or epiphytic.	Karst forest interior and margins. 330–600m. Frequent. (1; 6; 8; 7)
A. scandens (J. Sm. *ex* Fée) Mett.	*Barcelona et al. 2450*	Terrestrial and climbing.	Karst forest interior. Moist, waterlogged area. 330–560m. Rare. (1)
A. subnormale Copel.	*Barcelona et al. 2473*	Terrestrial.	Moist or waterlogged areas. 330–720m. Infrequent. (1; 6; 8)
A. tenerum G. Forst.	*Barcelona et al. 2449*	Epiphytic.	Moist or waterlogged area. 330—586m. Frequent. (1; 6; 8)
A. unilaterale Lam.	*Barcelona et al. 2446*	Terrestrial.	Moist or waterlogged areas. Infrequent. (1; 2; 8)
A. vittaeforme Cav.	*Barcelona et al. 2524*	Epiphytic.	Karst forest interior. 330–703m. Infrequent. (1; 3; 6; 2)
AZOLLACEAE			
Azolla pinnata R. Br.*	*Barcelona et al. 2614a*	Floating.	Rice paddies below limestone forest. 390–465m. Common. (8)
BLECHNACEAE			
Blechnum orientale L.*	not collected	Terrestrial.	Exposed areas, eroded roadcuts. 530–623m. Frequent. (6)
CYATHEACEAE			
Cyathea contaminans (Wall. *ex* Hook.) Copel.*	*Barcelona et al. 2553*	Terrestrial.	Karst forest interior and exposed, disturbed areas. 300–465m. Rare. (1; 8)
Cyathea sp.*	*Barcelona et al. 2521*	Terrestrial.	Karst forest interior. 543–586m. Frequent. (6)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
DAVALLIACEAE			
Davallia denticulata (Burm. f.) Kuhn var. *denticulata**	*Barcelona et al. 2682*	Terrestrial or epiphytic.	Reforested karst forests. 330–703m. Infrequent. (1; 3)
D. divaricata Blume	*Barcelona et al. 2536*	Terrestrial, epilithic, or epiphytic.	Karst forest interior. 480–720m. Infrequent. (6; 8)
D. falcinella (J. Sm.) C. Presl†	*Ramos BS42974* (UC)		(1)
D. pectinata J. Sm.*	*Barcelona et al. 2632*	Low-climbing epiphyte.	Summit of a very dry karst hill. ca. 525m. Seen only once. (8)
D. repens (L. f.) Kuhn†	*Ramos BS42970, BS43002* (UC)	Epiphytic.	(1)
D. solida (G. Forst.) Sw.*	*Barcelona et al. 2556*	Epiphytic.	Karst forest interior. 300–370m. Infrequent. (1)
D. trichomanoides Blume var. *lorrainii* (Hance) Holtt.	*Barcelona et al. 2554*	Epiphytic.	Karst forest interior. 300–370m. Infrequent. (6)
Davallodes hirsutum (C. Presl) Copel.*	*Barcelona et al. 2680*	Epiphytic.	Karst forest interior. 530–703m. Infrequent. (3;6)
DENNSTAEDTIACEAE			
Microlepia speluncae (L.) Moore	*Barcelona et al. 2441*	Terrestrial.	Karst forest margins, exposed thickets, abandoned farm. 543–623m. Frequent. (1; 6; 8)
Pteridium aquilinum (L.) Kuhn var. *wrightianum* (Wall. *ex* Agardh) Tryon [= *P. caudatum* (L.) Maxon?]	*Barcelona et al. 2552*	Terrestrial.	Disturbed, eroded, clayey/loamy (not limestone-derived) soil. 530–623m. Locally dominant. (2; 6)
EQUISETACEAE			
Equisetum ramosissimum Desf. ssp. *debile* (Vaucher) Hauke	*Barcelona et al. 2654*	Terrestrial.	Along Bugsoc River. 390–600m. Locally common. (1; 6)
GLEICHENIACEAE			
Dicranopteris linearis (Burm. f.) Underw. var. *subspeciosa* Holtt.*	*Barcelona et al. 2393*	Scrambling.	Roadcuts, exposed areas. Not limestone-derived soil. Infrequent. 560–585m. (4)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
D. linearis (Burm. f.) Underw. var.?*	*Barcelona et al. 2394*	Scrambling.	Roadcuts, exposed areas. Not limestone-derived soil. Infrequent. 560–585m. (4)
HYMENOPHYLLACEAE			
Cephalomanes atrovirens C. Presl*	*Barcelona et al. 2571*	Terrestrial or epiphytic.	In moist, shaded, waterlogged areas. 330–703m. Frequent. (1; 3; 8; 2)
Crepidomanes brevipes (C. Presl) Copel*.	*Barcelona et al. 2511*	Climbing epiphytes.	Karst forest interior. 330–720m. Infrequent. (1; 3; 6; 8)
C. humile (G. Forst.) Bosch*	*Barcelona et al. 2373*	Epiphytic.	Karst forest interior. (330–560m). Infrequent. (1)
Hymenophyllum polyanthos (Sw.) Sw.†	*Ramos BS43996, BS43043* (UC)	Epiphytic.	(1)
H. serrulatum (C. Presl) C. Chr.†	*Ramos BS43042* (UC, US)	Epiphytic.	(8)
LINDSAEACEAE			
Lindsaea ensifolia Sw. ssp. *ensifolia**	*Barcelona et al. 2616*	Terrestrial.	Karst forest interior. 390–600m. Rare. (6; 8)
L. repens (Bory) Thwaites var. *pectinata* (Blume) Mett. *ex* Kuhn forma *angusta* (Copel.) Kramer*	*Barcelona et al. 2541*	Epiphytic.	Karst forest interior. 330–703m. Rare. (1; 3; 6)
Sphenomeris retusa (Cav.) Maxon*	*Barcelona et al. 2396*	Terrestrial.	*Pteridium aquilinum*-dominated area in moist, non-limestone-derived soil. 560–585m. Rare. (4)
LOMARIOPSIDACEAE			
Bolbitis heteroclita (C. Presl) Ching*	*Barcelona et al. 2514*	Terrestrial, climbing, epiphytic, or epilithic.	Karst forest interioir. 330–720m. In moist areas. Common. (1; 6; 8)
Lomagramma copelandii Holtt.†	*Ramos BS42972* (UC)	Climbing or epiphytic.	**Endemic**. (1)
L. merrillii Holtt.*	*Barcelona et al. 2389*	Climbing.	**Endemic**. Karst forest interior. 620–720m. Rare. (6)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
L. pteroides J. Sm.*	*Barcelona et al. 2528*	Climbing.	**Endemic**. Karst forest interior. 510–546m. Frequent. (3; 6; 8)
Lomariopsis lineata (C. Presl) Holtt.*	*Barcelona et al. 2482*	Climbing.	Karst forest interior. 330–560m. Infrequent. (1)
Teratophyllum aculeatum (Blume) Mett. *ex* Kuhn*	*Barcelona et al. 2693*	Climbing or epiphytic.	Karst forest edge. 570–720m. Rare. (3; 6; 8)
LOXOGRAMMACEAE			
Loxogramme avenia (Blume) C. Presl*	*Barcelona et al. 2555*	Epiphytic.	Karst forest interior. 300–703m. Infrequent. (1; 3; 6; 8)
L. conferta (Copel.) Copel.†	*Ramos BS43037* (US)		
LYCOPODIACEAE			
Lycopodium cernuum L.	*Barcelona et al. 2395*	Terrestrial and scrambling.	Roadcuts and other exposed areas. 560–585m. Infrequent. (4)
MARATTIACEAE			
Angiopteris palmiformis (Cav.) C. Chr.*	*Barcelona et al. 2520*	Terrestrial.	Karst forest interior and margins. 543–586m. Frequent. (6)
A. pruinosa Kunze*	*Barcelona et al. 2491*	Terrestrial.	Karst forest interior, shaded. 543–586m. Frequent. (6)
OLEANDRACEAE			
Nephrolepis biserrata (Sw.) Schott	*Barcelona et al. 2443*	Terrestrial and scrambling.	Exposed thickets, abandoned farms. 330–560m. Frequent. (1; 8)
N. falcata (Cav.) C. Chr.*	*Barcelona et al. 2462*	Epiphytic.	Moist and shaded karst forest interior. 330–586m. Frequent. (1; 6)
N. multiflora (Roxb.) Jarrett *ex* Morton*	*Barcelona et al. 2589*	Terrestrial.	Disturbed, exposed areas. 570–600m. Common. (1; 3; 4; 6; 8; 2; 7)
Oleandra cf. *benguetensis* Copel.*	*Barcelona et al. 2698*	Epiphytic.	Karst forest interior. 600–703m. Rare. (3)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
OPHIOGLOSSACEAE			
Ophioglossum petiolatum Hook.*	*Barcelona et al. 2376*	Terrestrial.	Karst forest margin in clayey substrate. ca. 350m. Rare & seen only once.
PARKERACEAE			
Ceratopteris thalictroides (L.) brongn.*	*Barcelona et al. 2505*	Partially submerged.	In waterlogged areas. 543–586m. Locally common. (6)
POLYPODIACEAE			
Aglaomorpha acuminata (Willd.) Hovenkamp*	*Barcelona et al. 2513*	High canopy epiphyte.	Karst forest interior. 543–586m. Rare. (6)
A. heraclea (Kunze) Copel.*	*Barcelona et al. 2612*	High canopy epiphyte.	Karst forest interior. 543–703m. Infrequent. (3; 6; 8)
Belvisia mucronata (Fée) Copel.*	*Barcelona et al. 2527*	Epilithic or epiphytic.	Karst forest interior. 543–720m. Infrequent. (6; 8)
Drynaria descensa Copel.*	*Barcelona et al. 2679*	Epiphytic.	**Endemic**. Karst forest interior. 600–703m. Infrequent. (3)
D. quercifolia (L.) J. Sm.*	*Barcelona et al. 2636*	Epiphytic.	Karst forest interior. 445–525m. Common. (8)
D. rigidula (Sw.) Bedd.*	*Barcelona et al. 2644*	Epiphytic.	Karst forest interior. 445–525m. Rare. (8)
D. sparsisora (Desv.) Moore*	*Barcelona et al. 2709*	Epiphytic.	Karst Forest margin. 480–703m. Frequent. (3; 8)
Goniophlebium subauriculatum (Blume) C. Presl*	*Barcelona et al. 2678*	Epiphytic.	Karst forest interior. 600–703m. Locally frequent. (3)
Lecanopteris sinuosa (Wall. *ex* Hook.) Copel.*	*Dolotina et al.* s.n. (PNH)	High canopy epiphyte.	Karst forest interior. 600–703m. Seen only once. (3)
Lemmaphyllum accedens (Blume) Donk	*Barcelona et al. 2672*	Epiphytic.	Karst forest interior. 543–703m. Infrequent. (3; 6)
Lepisorus longifolius (Blume) Holtt.*	*Barcelona et al. 2706*	Epiphytic.	Karst forest margin. 480–600m. Seen only once. (8)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
Leptochilus macrophyllus (Blume) Noot. var. *fluviatilis* (Lauterb.) Noot.*	*Barcelona et al. 2497*	Terrestrial or epilithic.	Karst forest. Flowing, waterlogged forest. 330–586m. Rare. (1; 6)
L. macrophyllus (Blume) Noot. var. *macrophyllus*	*Barcelona et al. 2560*	Terrestrial or epilithic.	Karst forest interior. 300–600m. Frequent. (1; 8)
Microsorum commutatum (Blume) Copel.	*Barcelona et al. 2685*	Epiphytic.	Karst forest interior. 600–703m. Infrequent. (1; 3)
M. heterocarpum (Blume) Ching*	*Barcelona et al. 2595*	Epilithic.	Karst forest interior. 570–703m. Frequent. (3; 8)
M. longissimum Fée	*Barcelona et al. 2495*	Epiphytic.	Moist, shaded forest. 330–586m. Common. (1; 6)
M. membranifolium (R. Br.) Ching*	*Barcelona et al. 2557*	Terrestrial or low epiphyte.	Karst forest interior. 300–465m. Frequent. (1; 8)
M. monstrosum (Copel.) Copel.	*Barcelona et al. 2666*	Epiphytic and epilithic.	**Endemic.** Karst forest interior. 390–703m. Infrequent. (2; 3; 8)
M. punctatum (Blume) Copel.	*Barcelona et al. 2523*	Terrestrial or epiphytic.	Karst forest interior and disturbed areas. 330–703m. Frequent. (1; 3; 6)
M. rubidum (Kunze) Copel.	*Barcelona et al. 2487*	Terrestrial.	Exposed thickets, abandoned farm. 330–560m. Rare. (1)
M. samarense (J. Sm. *ex* C. Presl) Bosman*	*Barcelona et al. 2641*	Epiphytic, climbing, or epilithic.	**Endemic.** Karst forest interior. 445–525m. Locally frequent but only population seen. (8)
M. scolopendria (Burm.f) Copel.	*Barcelona et al. 2444*	Terrestrial.	Exposed thickets such as abandoned farms. 330–560m. Infrequent. (1)
M. zippelii (Blume) Ching*	*Barcelona et al. 2646*	Epilithic?	Karst forest interior. 575–600m. (8)
Platycerium coronarium (König. *ex* Müller) Desv.*	*Barcelona et al. 2624*	High canopy epiphyte.	Karst forest interior. 390–465m. Rare and seen only once. (8)
Pyrrosia lanceolata (L.) Farw.	*Barcelona et al. 2455*	Epiphytic on coconut tree.	Disturbed, exposed areas. 330–560m. Infrequent. (1)
P. longifolia (Burm. f.) C.V. Morton*	*Barcelona et al. 2629*	Epiphytic.	Karst forest interior and margins. 390–465m. Infrequent. (8)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
P. nummularifolia (Sw.) Ching†	*Ramos BS42994* (US)	Epiphytic?	
P. piloselloides (L.) M.G. Price†	*McGregor BS1268* (MICH)	Epiphytic?	
P. splendens (C. Presl) Ching	*Barcelona et al. 2575*	Epiphytic or epilithic.	**Endemic**. Karst forest interior and margins. 330–560m. Frequent. (1; 8)
Thylacopteris papillosa (Blume) Kunze *ex* J. Sm.	*Barcelona et al. 2539*	Epiphytic.	Karst forest interior. 530–703m. Infrequent. (1; 3; 6)
PSILOTACEAE			
Psilotum nudum (L.) Beauv.†	*Ramos BS42789*	Terrestrial.	(5)
PTERIDACEAE			
Pteris armata C. Presl*	*Barcelona et al. 2515*	Terrestrial.	Karst forest interior. 330–586m. Locally frequent. (1; 6)
P. ensiformis Burm.	*Barcelona et al. 2499*	Terrestrial.	Karst forest interior. (543-586m). Frequent. (1; 6)
P. mertensioides Willd.†	*Ramos BS43021* (NY, UC)		(1)
P. opaca (C. Presl) J. Sm. *ex* Fée	*Barcelona et al. 2653*	Terrestrial.	Along shaded riverbanks. 390–600m. Seen only once. (6)
P. oppositi-pinnata Fée*	*Barcelona et al. 2529*	Terrestrial.	Karst forest interior. 330–586m. Infrequent. (1; 6)
P. pellucida C. Presl	*Barcelona et al. 2615*	Terrestrial.	Karst forest margins, interior, and along trails. 330–560m. Infrequent. (1; 8)
P. philippinensis Fée*	*Barcelona et al. 2697*		Terrestrial. Karst forest interior. 330–703m. Infrequent. (1; 3)
P. tripartita Sw.*	*Barcelona et al. 2594*	Terrestrial.	Karst forest margins and other disturbed and exposed areas. 570–600m. Common. (8)
P. vittata L.	*Barcelona et al. 2464*	Terrestrial.	Along roadcuts and other exposed areas. 330–560m. Weedy. (1; 7)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
P. whitfordii Copel.	*Barcelona et al. 2591*	Terrestrial.	**Endemic.** Karst forest interior. 570–600m. Infrequent. (1; 8)
SCHIZAEACEAE			
Lygodium auriculatum (Willd.) Alston & Holtt.*	*Barcelona et al. 2533*	Scrambling.	Karst forest interior and exposed thickets. 543–586m. Common. (6)
L. circinnatum (Burm.) Sw.*	*Barcelona et al. 2488*	1	Scramblers in exposed thickets such as abandoned farms.
L. flexuosum (L.) Sw.*	*Barcelona et al. 2561*	1	Scramblers and thicket-forming in forest margins and other disturbed areas.
L. japonicum (Thunb.) Sw.*	*Barcelona et al. 2489*	1	Scramblers and thicket-forming in forest margins and other disturbed areas.
Schizaea inopinnata Selling†	*Ramos BS43015* (UC)	2	1000 ft. (2)
SELAGINELLACEAE			
Selaginella aristata Spring*	*Barcelona et al. 2409*	1	Epilithic on limestone walls along roadsides.
S. cupressina (Willd.) Spring	*Barcelona et al. 2642*	1; 6; 8	Terrestrial. Karst forest interior. Frequent.
S. delicatula (Desv.) Alston*	*Barcelona et al. 2429*	1	Terrestrial. Karst forest margins. Locally common.
S. engleri Hieron.	*Barcelona et al. 2525*	1; 6	Terrestrial. Karst forest interior and in waterlogged areas. Frequent.
S. flagellifera W. Bull*	*Barcelona et al. 2412*	Creeping on rockwall along road.	Karst forest margins or along trailsides. 330–560m. Infrequent. (1)
S. involvens (Sw.) Spring	*Ramos BS43012* (K, UC)	Terrestrial	(1)
S. llanosii Hieron.	*Barcelona et al. 2617*	Creeping on limestone wall.	**Endemic.** Karst forest interior. 330–465m. (1; 8)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
TECTARIACEAE			
Ctenitis boholensis Holtt.†	*Ramos BS42983* (TYPE - UC, K), *BS42984, BS43023*(UC)	Terrestrial.	**Bohol Endemic.** 1,000 ft. (1; 2)
C. humilis Holtt.	*Barcelona et al. 2656*	Terrestrial and epilithic.	**Endemic.** Shaded, clayey riverbanks and on rocks in damp forest. 390–600m. Rare. (6; 8)
C. pallens (Brack.) M. G. Price*	*Barcelona et al. 2609*	Terrestrial.	Karst forest interior. 570–703m. Infrequent. (3; 8)
C. silvatica Holtt.*	*Barcelona et al. 2545*	Terrestrial.	Karst forest interior. 530–623m. Infrequent. (3; 6)
Ctenitis sp.	*Barcelona et al. 2531*	Terrestrial.	Karst forest interior. 543–586m. Infrequent. (6)
Cyclopeltis presliana (J. Sm.) Berkeley	*Barcelona et al. 2435*	Terrestrial.	Karst forest interior, beneath boulders. 330–623m. Common. (1; 6; 8)
Heterogonium aspidioides C. Presl*	*Barcelona et al. 2598*	Terrestrial.	Karst forest interior. Waterlogged, moist, shaded areas. Karst forest interior. 570–600m. Locally common. (8)
H. pinnatum (Copel.) Holtt.*	*Barcelona et al. 2604*	Terrestrial.	Moist, waterlogged area. Karst forest interior. 330–600m. Frequent. (1; 8)
Pleocnemia irregularis (C. Presl) Holtt.*	*Barcelona et al. 2534*	Terrestrial.	Karst forest interior. 330–586m. Locally frequent. (1; 6)
P. presliana Holtt.	*Barcelona et al. 2418*	Terrestrial.	Karst forest interior. 330–560m. Locally frequent. (1)
Psomiocarpa apiifolia C. Presl*	*Barcelona et al. 2421*	Terrestrial.	**Endemic Genus.** Karst forest interior. Moist, limestone-derived soils. 330–586m. Locally infrequent. (1; 6; 8)
Pteridrys microtheca (Fée) C. Chr. & Ching†	*Ramos BS43025* (UC)	Terrestrial.	(2)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
Tectaria angulata (Willd.) Copel.*	*Barcelona et al. 2657*	Terrestrial.	Karst forest. Along riverbanks, loamy soil. 575–600m. Seen only in this locality. (6)
T. athyriosora M. G. Price	*Barcelona et al. 2627*	Terrestrial.	**Endemic.** Karst forest interior. 330–560m. Infrequent. (1; 8)
T. aurita (Sw.) S. Chandra*	*Barcelona et al. 2605*	Terrestrial.	Karst forest interior. Waterlogged or moist, shaded areas. 330–600m. Frequent. (1; 6; 8)
T. calcarea Copel.	*Barcelona et al. 2558*	Terrestrial.	**Endemic.** Shaded areas such as underneath limestone walls. 330–600m. Infrequent. (1; 8)
T. crenata Cav.	*Barcelona et al. 2637*	Terrestrial.	Karst forest interior. 445–703m. Locally common. (1; 3; 8)
T. decurrens (C. Presl) Copel.*	*Barcelona et al. 2530*	Terrestrial or epiphytic.	Karst forest interior. 330–586m. Infrequent. (1; 6)
T. devexa (Kunze *ex* Mett.) Copel.	*Barcelona et al. 2465*	Terrestrial.	Karst forest in moist, shaded areas, beneath boulders. 330–720m. Infrequent. (1; 6)
T. dissecta (G. Forst.) Lellinger	*Barcelona et al. 2607*	Terrestrial.	Karst forest margins. 330–703m. Infrequent. (1; 3; 8)
T. lobbii (Hook.) Copel. var. *lobbii*†	*Ramos BS43041* (GH, MICH, UC)	Terrestrial?	Sevilla River. 1,000 ft. (5)
T. melanocaula (Blume) Copel.*	*Barcelona et al. 2661*	Terrestrial.	Karst forest interior. 575–600m. Infrequent. (6)
T. ramosii (Copel.) Holtt.	*Barcelona et al. 2572*	Terrestrial.	**Endemic.** Karst forest interior. 330–560m. Infrequent. (1; 2; 8)
Tectaria cf. *villosa* Holtt.*	*Barcelona et al. 2613*	Terrestrial.	Karst forest interior. Waterlogged areas. 390–465m. Rare. (8)
THELYPTERIDACEAE			
Amphineuron immersum (Blume) Holtt.	*Barcelona et al. 2551*	Terrestrial.	Karst forest interior. 530–703m. Infrequent. (1; 3; 6; 8)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
A. terminans (Hook.) Holtt.*	*Barcelona et al. 2457*	Terrestrial.	Limestone-derived soil and swampy sinkholes. 330–586m. Infrequent. (1; 6; 8)
Chingia ferox (Blume) Holtt.	*Barcelona et al. 2651*	Terrestrial.	Along banks of Bugsoc River. 575–600m. Rare. (2; 6)
Christella parasitica (L.) Lév.*	*Barcelona et al. 2619*	Terrestrial.	Karst forest interior and margins. 390–465m. Infrequent. (8)
Macrothelypteris torresiana (Gaud.) Ching	*Barcelona et al. 2608*	Terrestrial.	Exposed thickets and trails. 390–600m. Frequent. (1; 8)
Pneumatopteris glabra (Copel.) Holtt.	*Barcelona et al. 2652*	Terrestrial.	**Endemic.** Along shaded riverbanks. 300–600m. (6; 5)
P. laevis (Mett.) Holtt*.	*Barcelona et al. 2683*	Terrestrial.	**Endemic.** Karst forest interior and margins. 600–703m. Infrequent. (1; 3)
P. ligulata (C. Presl) Holtt.*	*Barcelona et al. 2565*	Terrestrial.	Roadcuts and other exposed areas. 330–560m. Frequent. (1)
P. nitidula (C. Presl) Holtt.*	*Barcelona et al. 2610*	Terrestrial.	**Endemic.** Karst forest interior. 570–600m. Infrequent. (8)
Pronephrium asperum (C. Presl) Holtt.*	*Barcelona et al. 2400*	Terrestrial.	Karst forest interior. Moist, clayey soil. 543–586m. Infrequent. (6)
P. rhombeum (Christ) Holtt.*	*Barcelona et al. 2510*	Terrestrial.	Karst forest interior. 530–600m. Infrequent. (6)
P. ×xiphioides (Christ) Holtt.*	*Barcelona et al. 2509*	Terrestrial and epilithic.	**Endemic.** Karst forest interior and margins. 330–600m. Infrequent. (1; 6; 8)
Sphaerostephanos acrostichoides (Desv.) Holtt.	*Ramos BS42990* (BO, G, NY, UC, US); *Ramos BS42988* (GH, UC, SING)		600 m. (1)
Sphaerostephanos heterocarpus (Blume) Holtt.*	*Barcelona et al. 2426*	Terrestrial.	Karst forest margins. 330–560m. Frequent. (1)

TABLE 2. Continued.

FAMILY Species	Representative Vouchers (Herbaria)	Habit	Ecology, Frequency, and Location
S. productus (Kaulf.) Holtt.†	*Ramos BS42997* [BM; BO; SING; US]		
S. unitus (L.) Holtt.	*Barcelona et al. 2442*	Terrestrial.	Disturbed, exposed thickets such as abandoned farms. 330–560m. (1)
VITTARIACEAE			
Antrophyum reticulatum (G. Forst.) Kaulf.*	*Barcelona et al. 2516*	Epiphytic.	Karst forest interior. 543–586m. Infrequent. (6)
A. sessilifolium (Cav.) Spreng.*	*Barcelona et al. 2626*	Epiphytic.	Karst forest margins. 330–560m. Infrequent. (1; 8)
A. williamsii Benedict*	*Barcelona et al. 2568*	Epiphytic.	**Endemic.** Karst forest interior. 330–365m. Rare. (1; 6)
Vittaria elongata Sw.	*Barcelona et al. 2577*	Epiphytic.	Karst forest interior. 510–703m. Infrequent. (1; 3; 6; 8)
WOODSIACEAE			
Diplazium esculentum (Retz.) Sw.	not collected		Terrestrial. Weedy in exposed, disturbed areas.
D. maximum (Don) C. Chr.	*Barcelona et al. 2599*	Terrestrial.	Karst forest interior. Moist, clayey soil. 330–600m. Infrequent. (1; 6; 8)
D. pallidum (Blume) T. Moore*	*Barcelona et al. 2507*	Terrestrial.	Karst forest interioir. Moist, clayey soil. 300–586m. Infrequent. (1; 6)
D. petiolare C. Presl	*Barcelona et al. 2526*	Terrestrial.	Karst forest interior. Frequent. (1; 6; 8)
D. polypodioides Blume*	*Barcelona et al. 2675*	Terrestrial.	Karst forest interior. 600–703m. Infrequent. (3)
D. vestitum C. Presl†	*Cuming 349* (US)		**Endemic.**
Diplazium cf. *crenatoserratum* (Blume) T. Moore†	*Ramos BS42993, BS42998* (GH, UC, US)	Terrestrial?	(1; 8)

specimens (*Ramos BS42984*, UC and *Ramos BS43023*, UC) collected in Bilar and Dimiao in 1923.

The island of Bohol is the type locality of four other fern names. *Diplazium petiolare* C. Presl was first collected by Cuming in Bohol in 1841 and described as a new species in 1851. It was also later found in Luzon, Samar, Palawan, and Mindanao. The types of *Cyclosorus glaber* Copel. and *Thelypteris sevillana* Reed, [both synonyms of *Pneumatopteris glabra* (Copel.) Holtt.], a widespread Philippine endemic, were collected from Sevilla River in Bohol.

Our collections also include range disjunctions for three Philippine endemics, *Antrophyum williamsii* Benedict, *Drynaria descensa* Copel., and *Lomagramma merrillii* Hollt. The type of *Antrophyum williamsii* (*Williams 1579*, US), was collected in Baguio City, Benguet Province, northern Luzon in 1904. Other specimens that are doubtfully attributed to this species were *Elmer 10034* (MO), collected in the Cuernos Mountains in the island of Negros and *Copeland 1117* (cited in Copeland, 1905 as *A. parvulum*). Previously, *Drynaria descensa* was known only from Luzon with one collection (*Williams 1507*, US) from Lake Lanao, Mindanao. Until this survey, *Lomagramma merrillii* had been known only from five collections from Lake Lanao in Mindanao.

We also discovered wild populations of a species of staghorn fern, *Platycerium coronarium* (König. Ex Müller) Desv., a popular ornamental species in the Philippines. Unlike *P. grande*, a Philippine endemic staghorn that had been collected to extinction (i.e. extinct in the wild) for its ornamental value, wild populations of *P. coronarium* can still be found in the Philippines, although these are also vulnerable to extinction due to over collection. Eighteen previously reported species have not been recollected (Table 2).

Discussion and Conclusions

This survey provides baseline data for one of the most dominant elements of the understory cover in the limestone forests of Bohol, and particularly for RSPL. It provides new information on species composition and increases our understanding of species associations in these unique habitats. The karst forests of Bohol remain one of the most intact, lowland, old secondary growth examples in the country. Although the total number of species so far identified is seemingly low, the associations of taxa adapted to the limestone substrate is quite unique and is not comparable to the more diverse high elevation montane forests. The genera *Asplenium*, *Selaginella*, *Microsorum*, *Pteris*, and *Tectaria* are the most diverse in species composition. *Asplenium* is the most species-rich, and includes both infrequent, locally common, and habitat-specific taxa. Sterile juvenile plants of *Asplenium scandens*, for instance, were found only in Sitio Duangon in Bilar and nowhere else in RSPL. The epiphytic *Lepisorus longifolius* (Blume) Holtt. is locally common in the forest margin in La Victoria, Valencia but found nowhere else in RSPL. Likewise, the high canopy epiphyte staghorn, *Platycerium coronarium*, was only found in the karst forest of Barangay Marawis, Valencia. The abundance of *Selaginella* (7 species) is quite remarkable in that this genus sometimes constitutes more than 80 percent of the

herbaceous understory cover in some portions of the forest. The apparently low representation of the Hymenophyllaceae (filmy ferns) and the total absence of the Grammitids, two of the most species-rich families in the moist montane forests, indicates a generally dryer environment year round.

We were also able to determine and confirm the ecological and conservation status of some of the noteworthy species of pteridophytes in Bohol especially the endemics and those whose conservation status are indeterminate due to deficient collection and ecological data. For instance, *Ctenitis humilis*, a local endemic known only from two collections from Bohol and Mindoro before this survey, is now known to be rare in its natural habitats, i.e. along a very short strip of a loamy riverbank, on moist boulder in forest interior, and on steep cliffs. *Ctenitis boholensis*, on the other hand, a Bohol Island endemic known only from three collections in Bilar and Dimiao in 1923 has not been rediscovered. Despite their rarity, neither *C. humilis* nor *C. boholensis* is classified in the threatened categories of IUCN. Likewise, *Antrophyum williamsii* Benedict, the type, (*Williams 11579*, US) of which came from Baguio, Benguet Province in northern Luzon, is a new record for Bohol. Specimens doubtfully attributed to this species were collected from Mt. Apo (*Copeland 1117* in Polyp. Phil. 109. 1905) and Cuernos Mountains (*Elmer 10034*, MO and *Elmer 10031*, NY) in Negros a hundred years ago. Previously reported by Copeland (1905) as *A. parvulum*, these specimens may indeed be *A. williamsii*, as Copeland (1961, p. 546) himself mistrusts his previous report because immature or dwarfed individuals of several species may look unlike the species in full development, and more or less resemble each other. We agree with Copeland.

Although collected in sterile condition, another Philippine endemic fern, *Drynaria descensa* Copel., is also a new record for Bohol. Previously, all but one specimen (*Williams 1507*, US from Lanao del Sur in Mindanao) of *D. descensa* have been collected from Luzon. The type is from Muñoz, Nueva Ecija Province in Luzon [*Copeland PPE42* (holotype: MICH; isotypes: B, BM, G, K, L, MU, NY, S, NSW, UC)]. The collection from Bohol therefore indicates that *D. descensa* may have a wider distribution, from Luzon to Mindanao, and not necessarily be a disjunct as the meager collection data suggest. Before our survey, the endemic *Lomagramma merrillii* had been known only from collections from the provinces of Lanao del Sur and Davao del Sur, in Mindanao, hence, the population in Sierra Bullones in Bohol represents the northernmost extension of its range.

Noteworthy non-endemic species have also been collected in Bohol. *Taenitis cordata* (Gaud.) Holtt., a species represented by only a few collections from limestone substrates in the Philippines (e.g. Dimiao, Bohol. 1923, *Ramos BS43013*, MICH, UC & US, and Samar Island. 1971, *Colina 454*, CEBU) was recollected from Barangay Marawis, Valencia. Our collection (*Barcelona et al. 2618*) represents the first specimen of this species at the PNH. Plants of *Lindsaea ensifolia* Sw. ssp. *ensifolia* were found to occur very sparsely in Bugsoc, Sierra Bullones and Marawis, Valencia. Although this subspecies is widely distributed in the world (see Holttum, 1971) and found throughout

Malesia, it is locally rare in the Philippines, only represented by a few collections from Luzon and Sibuyan with the most recent specimen collected in 1950 on Guimaras Island (*Sulit PNH 12622*, MICH). No specimens of this collection or of any other collections of this species were at the PNH.

We were not able to rediscover populations of 17 fern species previously reported in Bohol. *Davallia falcinella* (J. Sm.) C. Presl, a widespread semi-endemic that is also reported from the Marquesas was collected by Ramos in Bilar in 1923. Likewise, *Tectaria lobbii* (Hook.) Copel. is only represented in the Philippines by a single collection of var. *lobbii* from Sevilla River in 1923 (*Ramos BS4301*, GH, MICH, UC); this variety has also been reported from Sarawak in Borneo. *Tectaria lobbii* (Hook.) Copel. var. *denticulata* Holtt. is currently known only from the type collection from Sarawak and *T. lobbii* (Hook.) Copel. var. *allosora* Holtt. has been reported from the Moluccas (Holttum, 1991). On the other hand, *Pteris opaca* (C. Presl) J. Sm. ex Fée, a widely collected semi-endemic (also reported from Celebes) is locally rare in Bohol, having been first collected in Sevilla River in 1923 (*Ramos BS43040*, US). Recently, we found another population of this species in a similar habitat, along the Bugsoc River in Sierra Bullones at 390–400m (*Barcelona et al. 2378*, PNH).

Our recent rediscovery of rare fern endemics in the wild as well as wild populations of species of high ornamental value such as the staghorn *Platycerium coronarium*, *Asplenium* spp., *Drynaria* spp., *Microsorum* spp., and *Lepisorus longifolius*, implies that the karst forests of RSPL and adjacent areas are still considerably pristine. Bohol is one of the few provinces in the Philippines where ferns have not yet been collected on a commercial scale for their aesthetic value.

The presence of many non-governmental organizations (NGOs) in Bohol working on several conservation-related projects does have a significant impact on conservation. The cooperation between the NGOs, the local government units (LGUs), government agencies, local offices of the Department of Environment and Natural Resources (DENR), and the Department of Tourism is admirably incomparable to other parts of the Philippines. Because of this, the *Boholanos* (people of Bohol) have been able to successfully maintain the integrity of their forests. Despite the potential for economic development in the island, Bohol has capitalized on its rich biodiversity and natural geologic wonders (caves and karst hills) for cash sources through ecotourism. In fact, Bohol Island was awarded the coveted "Galing Pook Award" (= "Good Place" Award) in 2004 for its Ecotourism Development Program. This award recognizes local governments in the Philippines that demonstrate excellence in governance.

Acknowledgments

This initiative is a collaborative effort of the Philippine National Herbarium (PNH), National Museum of the Philippines and the *Boholanos* (local people of Bohol) with financial support from the Ford Foundation, U.S.A. and the European Commission through the Soil and Water

Conservation Foundation (SWCF), Inc.

This study has not been possible without the support of several institutions, offices, and persons to which we are most grateful. We are grateful to the Protected Areas Management Board of RSPL, DENR regional office in Cebu and local offices in Tagbilaran and Bilar in Bohol for endorsing the project, the local governments, especially the mayors and the Barangay Captains in the municipalities of Bilar, Garcia Hernandez, Guindulman, Sierra Bullones, and Valencia for the warm hospitality during our short stay in their communities. We appreciate the field assistance of our local guides and student volunteers.

Literature Cited

Amoroso, V. B., F. M. Acma and H. P. Pava. 1995. *Diversity, status, and ecology of pteridophytes in selected forests in Mindanao.* CMU Publications Office, Mindanao. 403pp.

Barcelona, J. F. 2002. Philippine pteridophyte collections as a resource for conservation planning. Proceedings of the "International Symposium on the Fern Flora Worldwide: Threats and Responses" sponsored by the British Pteridological Society and IUCN- Species Survival Commission." Fern Gaz. 16:307–312.

Barcelona, J. F. 2003. The taxonomy and ecology of the pteridophytes of Mt. Iraya & vicinity, Batan Island, Batanes Province, Northern Philippines. Pp. 299–325, in Chandra and Srivastava (eds). *Pteridology in the New Millenium.* Kluwer Academy Publishers. The Netherlands: 299–325.

Barcelona, J. F. 2003. Preliminary report on the ferns and ferns allies (pteridophytes) of Mt. Bali-it, Balbalasang-Balbalan National Park, Kalinga, northern Luzon, Philippines. Sylvatrop, the Technical Journal of Philippine Ecosystems and Natural Resources 13:81–92.

Barcelona, J. F. 2004. Collection and conservation status of pteridophytes in Panay Island, Philippines. Philipp. Scient. 41:57–73.

Copeland, E. B. 1908. Pteridophytes of the Horn of Negros. Leaflets of Phil. Bot. 2:387–425.

Copeland, E. B. 1910. The ferns of Mt. Apo. Leaflets of Phil. Bot. 3:791–851.

Hatusima, S. 1966. An enumeration of the plants of Batan Island, N Luzon, Philippines. Mem. Fac. Agr. Kagoshima Univ. 5:13–70.

Holttum, R. E. 1971. *Lindsaea*-group. Fl. Males. I, 3:177–254.

Holttum, R. E. 1991. *Tectaria* group. Fl. Males. II, 2(1):1–132.

Iwatsuki, K. and M. G. Price. 1977. The pteridophytes of Mt. Burnay and vicinity, northern Luzon. Tonan Ajia Kenkyu (Southeast Asian Studies) 14(4):540–572.

Merrill, E. D. 1908. On a collection of plants from the Batanes and Babuyanes Islands. Phil. J. Sci. 3:385–442.

American Fern Journal 96(1):21–30 (2006)

Leaf Phenology of the Climbing Fern *Lygodium venustum* in a Semideciduous Lowland Forest on the Gulf of Mexico

KLAUS MEHLTRETER

Departamento Ecología Funcional, Instituto de Ecología, A.C., km 2.5 antigua carretera a Coatepec No. 351, Congregación El Haya, Xalapa 91070, Veracruz, México

ABSTRACT.—Leaf phenology in a population of the climbing fern *Lygodium venustum* was observed during a 31-month period in Veracruz, Mexico. The study site is located 100–200 m from the Gulf coast in the understory of a semideciduous lowland forest dominated by trees of *Enterolobium* and *Ficus*. Four leaf parameters: leaf growth of main and secondary axes, number of living leaves, leaf production and leaf mortality were scored monthly and correlated with two climatic factors: monthly mean temperature and precipitation. Sixty percent of the 37 individuals were supported on lianas, dead wood or shrubs. Smaller, unsupported plants with a height of less than 2.5 m had a mean number of 1.9 ± 0.27 leaves and produced 3.7 ± 0.52 leaves per year and did not become fertile. Over 50 % of the leaves died within the first 3 months, whereas over 10% lived for 12 to 30 months, resulting in a mean leaf life span of 5.6 ± 0.7 months. All leaf parameters were seasonal with the exception of the growth of the main axes. During the rainy season, leaf growth of secondary axes and leaf mortality increased approximately threefold and leaf production rose twofold. Correlations between climatic factors and leaf parameters were strongest within a time lag of one month. The strongest correlation was found between precipitation and the growth of secondary axes, indicating that water is the limiting factor. One to five dormant buds developed on 17.7% of the leaves contributing to 40.3% of the total leaf growth. The potentially long life span of the climbing leaves and the outgrowing dormant buds make this species a successful pioneer in disturbed vegetation and a competitive weed in Mexican vanilla plantations.

Ferns are rarely considered as study organisms for phenological research in the tropics (Mehltreter and Palacios-Rios, 2003), because trees and shrubs are of more commercial interest, and because deciduous tree species are responsible for considerable microclimatic changes within a forest (Lieberman, 1982; Bullock and Solis-Magallanes, 1990; Foster, 1996; Williams-Linera, 1999). On the other hand, ferns and other understory perennial herbs must adapt to the changing microhabitat conditions created by deciduous canopy species. For example, the smaller root system of ferns might have more difficulty accessing water during the dry season. Because ferns are independent of pollinators for reproduction and animal vectors for their dispersal (Barrington, 1993) it might be expected that their rhythms of leaf growth and fertility are primarily responding to seasonal changes in precipitation and temperature.

Prior to the introduction of the Asian species *Lygodium microphyllum* (Cav.) R. Br. into Florida where it has become an invasive weed, there was no published data on the life history of any *Lygodium* species. Recent research on the genus has focused on paleontological (Collinson, 2002), morphological-anatomical (Carlquist and Schneider, 1998), developmental (Mendoza *et al.*, 1998) and physiological aspects (Kurumatani *et al.*, 2001), but no ecological

studies have been published. Consequently, the present contribution represents the first ecological field study in a species of this genus. Its objective was to measure leaf growth, leaf production, leaf mortality and fertility of a natural population of *L. venustum*; to determine their seasonality, leaf life span and the frequency of different orders of ramification; to correlate these results with monthly means of temperature and precipitation; and to relate these findings to a potential weedy character.

Lygodium (Schizaeaceae) is a widely distributed genus of about 35 species, with three native species in Mexico: *L. heterodoxum* Kunze, *L. volubile* Sw. and *L. venustum* Sw. (Mickel and Smith, 2004). *Lygodium venustum* is widely distributed in Latin America, from Mexico to Paraguay and the Caribbean islands, where it grows from sea level to 1100 m elevation. It is one of about 200 climbing fern species (Mehltreter, 2002) with specialized adaptations for a liana life-form. While some ferns (e.g. *Bolbitis bernoullii* (Kuhn *ex* Christ) Ching, *Lomariopsis recurvata* Fée) climb with their rhizomes and others (e.g. *Hypolepis nigrescens* Hook.) use their spreading leaves to find and hold on to the surrounding vegetation, *L. venustum* is a true climber with a twining rachis. Like those of *L. japonicum* (Thunb.) Sw., the leaves of *L. venustum* are produced from a subterranean rhizome in a heteroblastic series (Mueller, 1982b), i.e. the first juvenile leaves are dichotomously to anisotomously divided and of determinate growth of up to 20 cm (K. Mehltreter, pers. obs). Larger, twining leaves are 3–4 pinnate, of indeterminate growth and can reach a length of 10 m or more. Leaf rachis elongation near the leaf tip is rapid, whereas pinnae expand tardily. The pinnae consist of a short axis bearing two opposite, lateral, pinnate-pinnatifid pinnules and a terminal dormant bud; the latter can develop, in response to injury or destruction of the main leaf tip by herbivores or fungi (Mueller, 1983), into a ramifying leaf blade. Outgrowing buds of the primary pinnae do not enlarge just the pinnae axis to produce more pinnules, rather resume the same morphological ramification pattern as an entire leaf. Consequently, the buds of the primary pinnae become, structurally, a new "rachis" (additional levels called secondary, tertiary, etc. axes to avoid terminological confusion) with new pinnae that again bear leaf buds, but it is unknown how often this ramification can be repeated under natural conditions. The stipe and rachis have a diameter of 1–3 mm and are morphologically unable to thicken secondarily.

Materials and Methods

This study was conducted in a semideciduous coastal lowland forest of the Biological Station of La Mancha on the Gulf coast of the state of Veracruz, Mexico (19°36′00″N, 96°22′40″W). The forest canopy, which reaches a height of up to 22 m, is dominated by large individuals of *Brosimum alicastrum* Sw., *Bursera simaruba* (L.) Sarg., *Cedrela odorata* L., *Enterolobium cyclocarpum* (Jacq.) Griseb., *Ficus cotinifolia* Kunth and *Ficus obtusifolia* Kunth. Common species in the middle stratum are *Coccoloba barbadensis* Jacq., *Jacquinia macrocarpa* Cav., *Nectandra salicifolia* (Kunth) Nees, *Piper amalago* L. and

Randia monantha Benth. Lianas, e.g., *Agdestis clematidea* DC., *Paullinia tomentosa* Jacq. and *Vitis bourgaeana* Planch, are frequent, but epiphytes are very rare (Castillo-Campos and Medina A., 2002). The climate is characterized by a mean annual temperature of 25.6°C, a mean annual precipitation of 1198 mm, and a dry season from November to April, when mean precipitation is less than 45 mm per month. A deep, sandy clay soil is developed on old sand dunes (Geissert and Dubroeucq, 1995).

In January 1999, all 37 individuals of *L. venustum* encountered within an area of 150 × 50 m were tagged. Height of the *Lygodium*-plants, the distance from soil to the uppermost part of the plant, was measured with a metric tape or in larger plants with a 12 m telescopic measuring rod. The support type was classified within four categories: no support, liana, shrub, and dead wood. The diameter of the climbing support (i.e. liana, branch, or twig) that was entwined by *Lygodium*-leaves was also recorded. Twenty-two of these 37 plants were chosen for the phenological study because they were accessible by a ladder, i.e. plants that grew nearby to a tree to access the larger leaves without damaging them by leaning a ladder on them, or that were less than 2.5 m high. From February 1999 to August 2001, all new leaves were tagged, counted and measured monthly. Apical and lateral leaf growth of each order of ramification was computed for each plant as the number of centimeters added to all developing leaves between monthly observations. Leaf production was determined as the number of new leaves produced by a plant in one month. Leaf mortality was determined as the number of dead (without green blades) or lost leaves on a plant during one month, counting the mortality of each leaf only once. The mean leaf number for the population was calculated as the average of living leaves per plant. Leaf life span was calculated as the difference between the date of first observation of occurrence and the date of death or disappearance of that same leaf. Monthly leaf production and growth rates were extrapolated by multiplying the results by 30 and dividing by the number of days of each observation period. Linear correlations between monthly means of temperature, precipitation and phenological parameters were tested for their level of significance. Climatic data are from the nearby Biological Station of La Mancha. Statistical analyses were performed with *SigmaStat* (1995). Applied tests and correlations are cited in the text, tables and figure legends. Means are listed with ± 1 SE. Species vouchers (*Mehltreter 1133, 1134*) were deposited at the herbarium of the Instituto de Ecología, A. C. in Xalapa (XAL).

Results

Thirty-seven individuals clumped in 12 groups were found in the study plot of 7500 m^2 (= 49 plants·ha^{-1}). 40.5% of the plants were less than 50 cm in height and had no support for their climbing leaves (Fig. 1). All larger plants climbed on one of the support categories: woody lianas (24.3%), dead wood, i.e. fallen branches and twigs (24.3%) or shrubs (10.8%). The mean diameter of the embraced support (i.e. liana, branch or twig) was 1.05 ± 0.12 cm (n = 33 leaves). Only two plants reached more than 5 m in height.

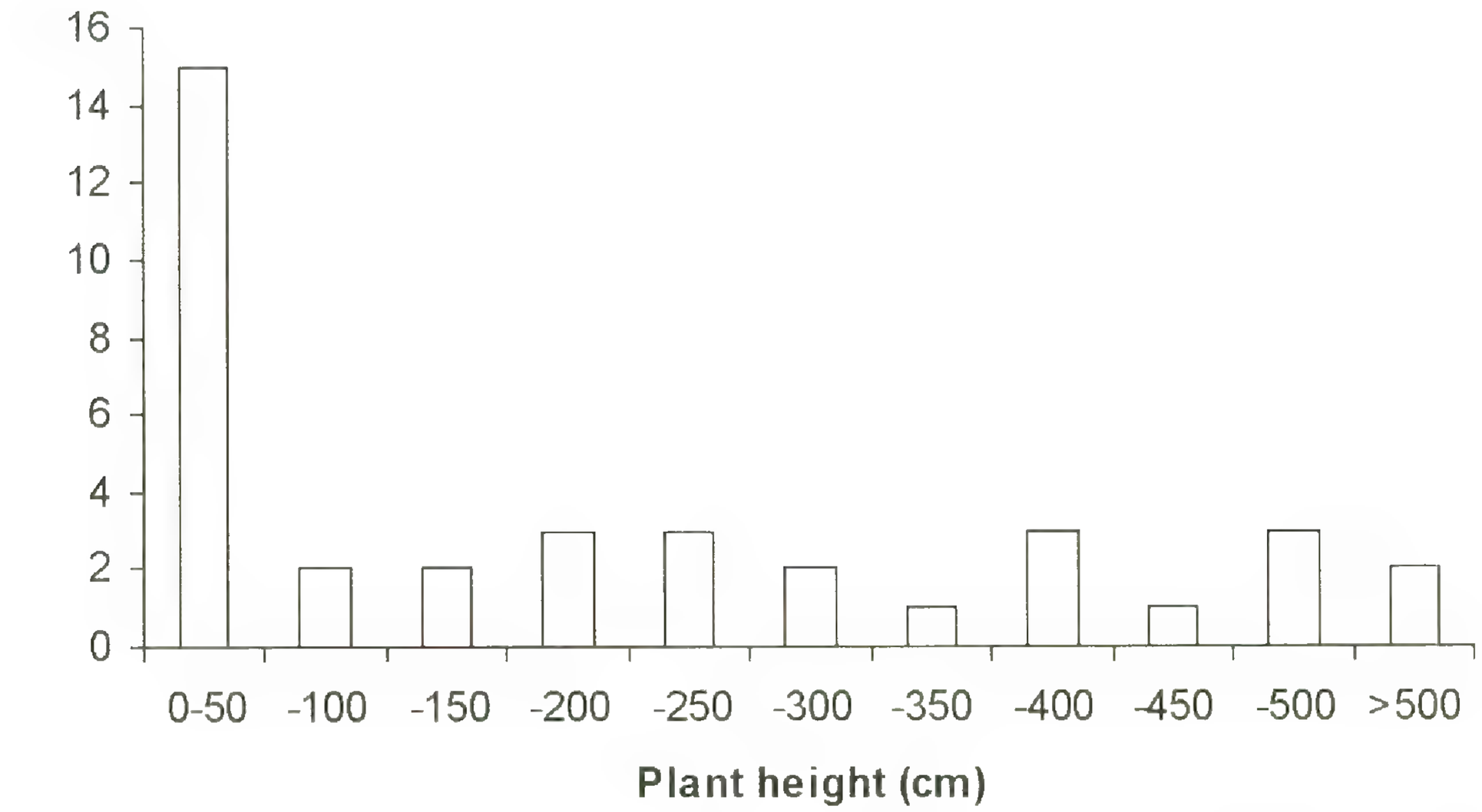


FIGURE 1. Plant height of a population of *Lygodium venustum* (n = 37) in La Mancha. Only plants of the first height class (0–50 cm) had no climbing support.

The mean leaf number of the population was 1.9 ± 0.27 per plant (n = 22). Leaves were produced at a rate of 3.7 ± 0.52 leaves per year, and died at a rate of 3.8 ± 0.63 leaves per year (Fig. 2). Their mean leaf life span was 5.6 ± 0.7 months. However, 54.3% of the leaves died within the first 3 months, whereas

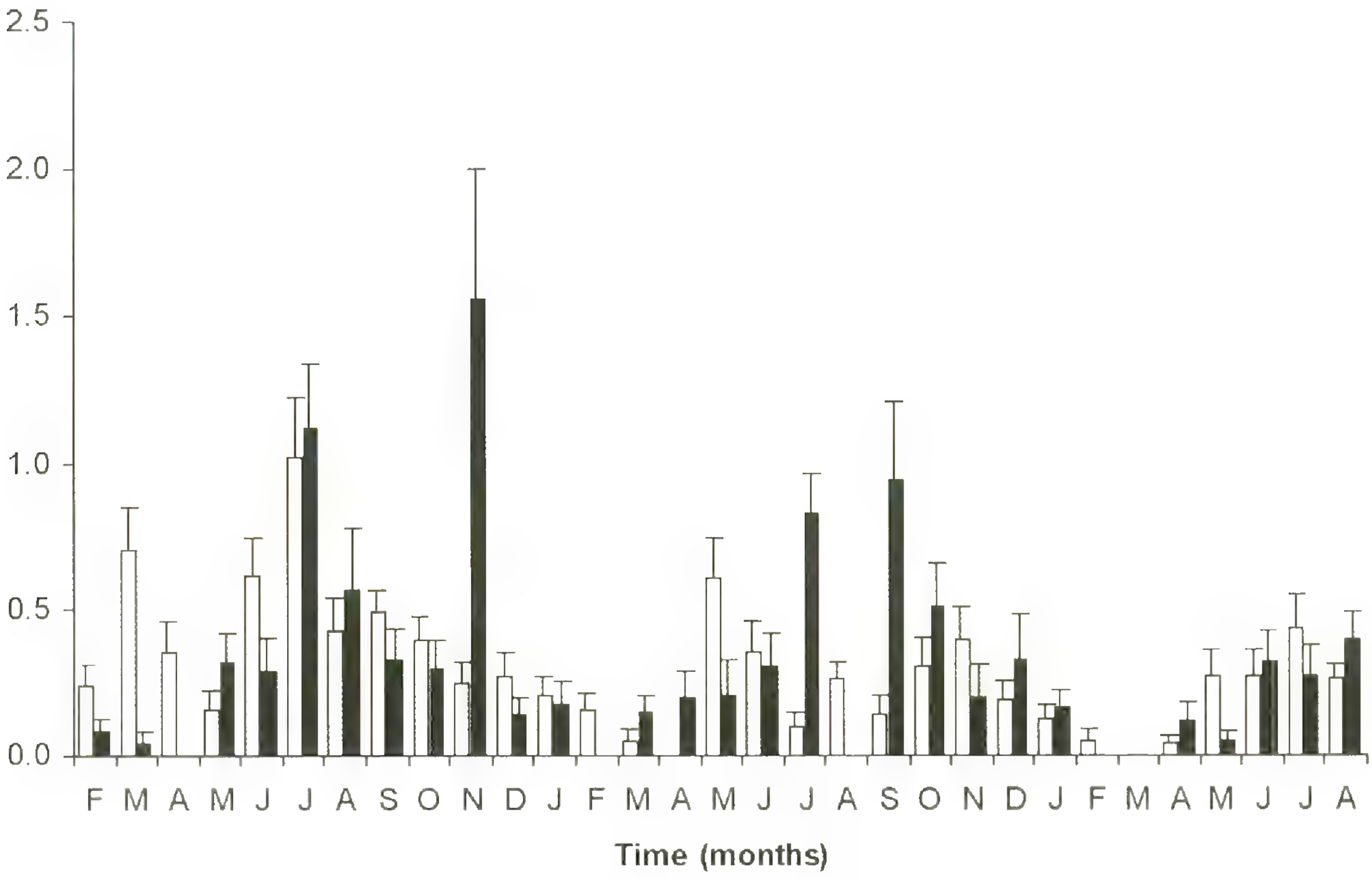


FIGURE 2. Monthly mean leaf production (white bars) and leaf mortality (black bars) of *Lygodium venustum* in La Mancha from February 1999 to August 2001. Means ± 1 SE, n = 22.

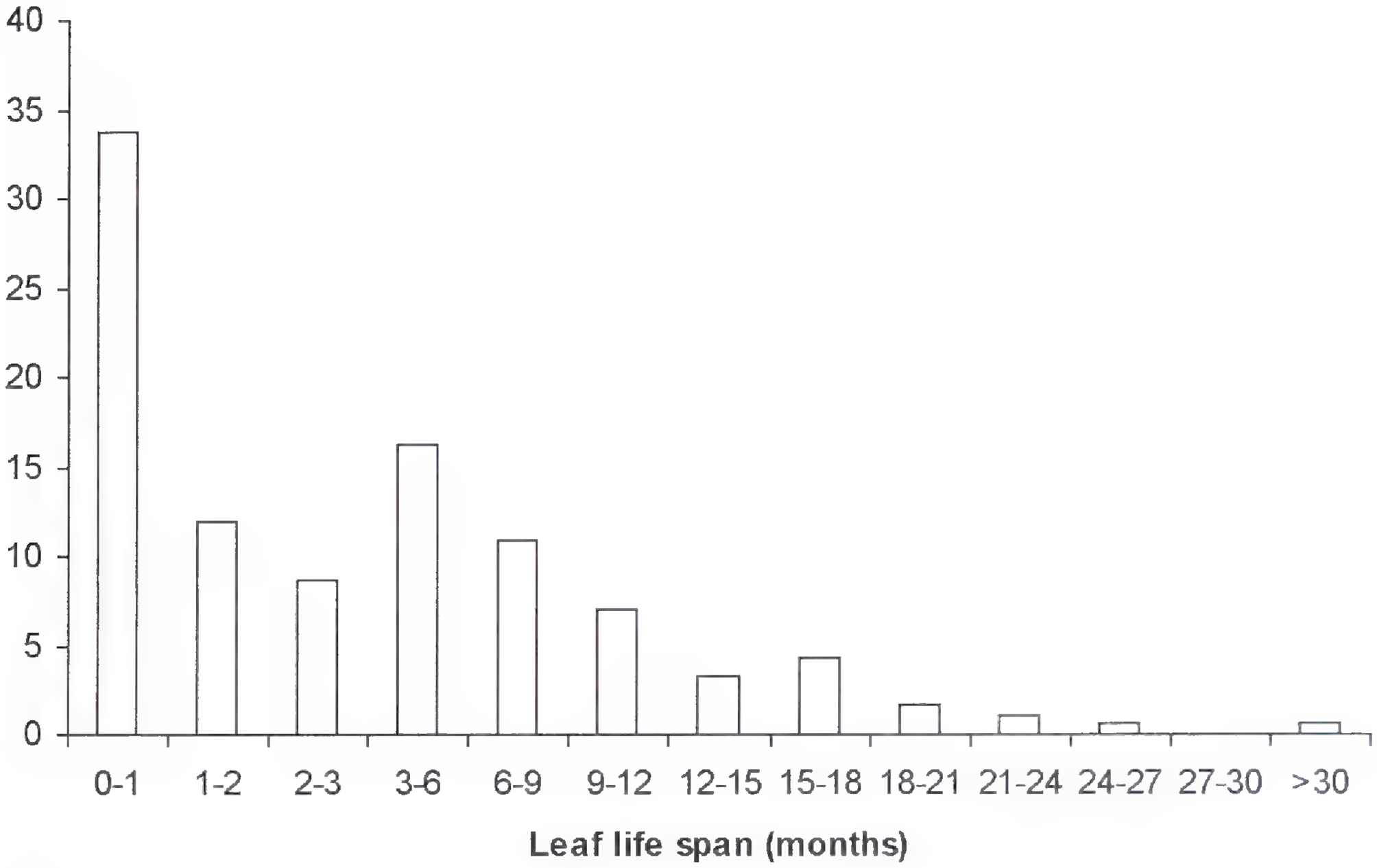


FIGURE 3. Distribution of the leaf life span of 209 leaves from 22 plants of *Lygodium venustum* in La Mancha.

11.4% lived between 12 and 30 months (Fig. 3). One leaf was alive during the entire observation period of 31 months. However, the leaf blade of the main rachis of this leaf did not stay intact, but was replaced by the leaf blades of emerging secondary axes. Leaf life span was strongly positively correlated with leaf length ($r = 0.48$, $P < 0.001$, $N = 184$). Mean leaf growth was 20 ± 5.1 cm per month. The most vigorous plant grew 85 ± 2.7 cm per month. On 17.7% of the leaves, one to five lateral buds of different levels grew out (buds of secondary axes, i.e. pinnae 11%, tertiary axes 4.3%, quaternary axes 1.4% and quinternary axes 1.0%). Such outgrowth of the lateral buds contributed 40.3% to the total leaf growth. The mean leaf length (stipe and main rachis) was 39 ± 5.7 cm. Of the 209 leaves observed, 70.3% were shorter than 50 cm, 13.4% were 50–100 cm long, 13.4% were 100–200 cm long and 2.9% were 200–600 cm long. No leaves became fertile during the observation period.

Most leaf parameters were significantly correlated among each other and with mean temperature and precipitation (Table 1, Fig. 4). Correlation coefficients between leaf parameters and precipitation were higher than between leaf parameters and mean temperature. The strongest correlation existed between leaf growth of secondary axes and precipitation. The application of a time lag series with a one-month delay increased the correlation coefficient between leaf parameters and climatic factors with the exception of leaf production and precipitation (Table 1). With a two-month delay, all correlation coefficients became smaller with the exception of the correlation between leaf mortality and mean temperature ($r = 0.69$, $P = 0.001$).

TABLE 1. Correlation coefficients between monthly population means of leaf parameters of *Lygodium venustum* in La Mancha, Gulf of Mexico, and monthly means of climatic factors during the 31-month observation period. Results of a time lag series with one month delay are given in parentheses.

	Growth (sec. axes)	Leaf production	Leaf mortality	Mean temperature	Precipitation
Growth (rachis)	0.51**	0.62***	0.39*	— (—)	— (0.46**)
Growth (secondary axes)		—	0.36*	0.42* (0.56**)	0.60*** (0.70***)
Leaf production	0.36*	0.37* (0.46**)		0.45* (—)	
Leaf mortality			— (0.56**)		— (0.64**)

—, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

Although leaves were produced and died during both seasons (Fig. 2), all leaf parameters differed significantly between seasons ($P < 0.05$, $df = 21$, Table 2), with the exception of leaf growth of the main rachis. Leaf growth of secondary axes and leaf mortality increased approximately threefold, and leaf production rose twofold during the rainy season from May to October (Table 2).

DISCUSSION

The studied population of *Lygodium venustum* was highly aggregated with groups of a few large plants climbing on other lianas or dead wood and several small plants without a climbing support. Nevertheless all plants were separated from each other by at least 20 cm distance. This distribution pattern should be

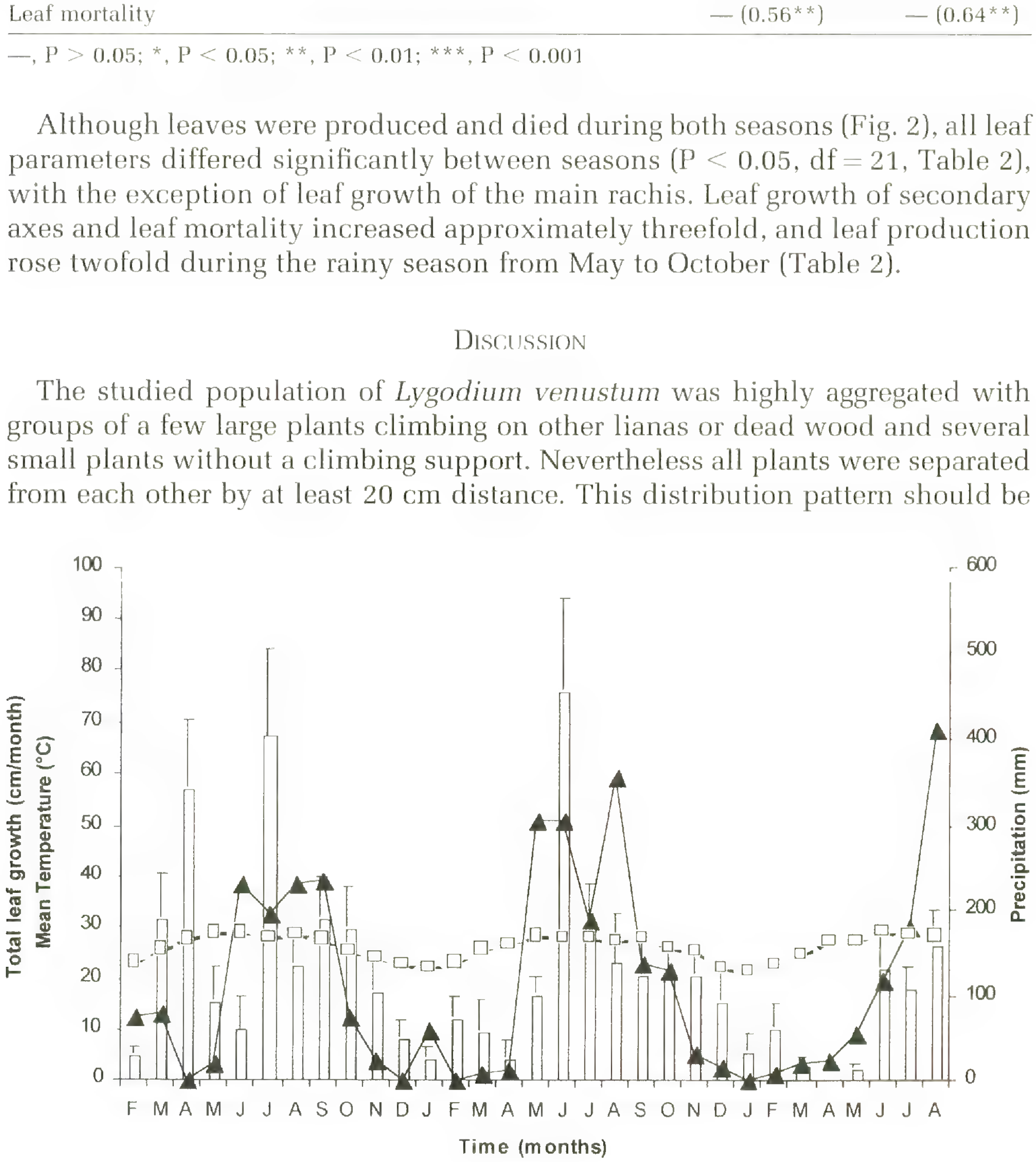


FIGURE 4. Total leaf growth (main rachis and secondary axes) of *Lygodium venustum* (bars. Means + 1 SE, n – 22), monthly precipitation (solid triangles) and mean temperature (open squares) in La Mancha from February 1999 to August 2001.

TABLE 2. Seasonality of leaf parameters of *Lygodium venustum* in La Mancha, Gulf of Mexico, during the 31-month observation period (paired t-test, df=21). Secondary axis growth was compared with a Wilcoxon signed rank test, because data were not normally distributed. Means ± 1 SE.

	Dry season (Nov–Apr)	Rainy season (May–Oct)
Rachis growth (cm)	10.3 ± 3.05	14.7 ± 3.62
Secondary axis growth (cm)	3.57 ± 1.66*	12.5 ± 4.02*
Leaf production	0.20 ± 0.04**	0.38 ± 0.06**
Leaf mortality	0.14 ± 0.04**	0.39 ± 0.06**

*, $P < 0.05$; **, $P < 0.001$

expected for a pioneer species that germinates and colonizes clearings and disturbed areas where broken tree branches and lianas are frequent. The presence and persistence of small plants for over 30 months within the understory indicates a capacity for this species to maintain a living reserve awaiting presumably better light conditions similar to the persistent seedlings of several tree species.

Species of *Lygodium* have been reported as weedy and as invaders in abandoned coconut and banana plantations (Tryon and Tryon, 1982). The Old World species *Lygodium japonicum* and *L. microphyllum* were introduced in Florida, where the latter readily invades disturbed areas (Nauman and Austin, 1978; Pemberton and Ferriter, 1998). In 1999, it was estimated that L. microphyllum covered 107,000 acres in Florida (Langeland, 2002). Because its extensive growth presents a fire hazard, biologic control organisms have been sought (Mound, 2002).

In Mexican vanilla plantations, *L. venustum* often becomes weedy, because it finds the right light conditions and a perfect support plant (J. G. García-Franco, pers. comm.). The rhizome of *L. venustum* grows about 10 cm below soil level, branches dichotomously several times and produces new leaves near each branch tip (K. Mehltreter, pers. obs.). Plants are difficult to eradicate because when pulled, rhizomes fragment and the remaining branch tips readily regenerate new leaves. In laboratory cultures, *L. venustum* needs about three months to develop from spore to the production of a first sporophyte leaf (Mendoza *et al.*, 1999). Therefore, it cannot be considered a rapidly developing species, because its life cycle needs too much time. It would only have a chance to be a successful pioneer, if it could maintain a reserve of young sporelings in the understory. However, during the study period, neither gametophytes nor new young sporophytes were observed. This suggests that sporelings probably develop more often in newly exposed, open sites rather than in the shady understory.

It was technically impossible to take into account the largest plants on the study site (see Methods). For this reason, the results presented allow only an approximation of the leaf parameters of larger adult plants of *Lygodium venustum*. The observed smaller plants had on average two leaves and a leaf turnover of six months. The relatively short life span of the leaves must be interpreted cautiously, because most leaves died as a consequence of mechanical damage or herbivory within the first three months. The potentially most important herbivore is the land crab *Gecarcinus lateralis* (Gecarcinidae) that

frequents the *Lygodium* sites and often has caused damage to the flagging tape as well (K. Mehltreter, pers. obs.). However, larger leaves had significantly longer life spans ($r = 0.48$, $P < 0.001$, $N = 184$) of 12–31 months (Fig. 3), similar to wet forest, shade-tolerant trees (32.2 months, Coley and Aide, 1991). Longer leaves of *L. venustum* have the advantage that their sprouting dormant buds can climb the same support as the primary rachis, whereas new leaves must first find their own climbing support. For this reason dormant buds are more successful and their growth made up 40.3% of the total leaf growth although only 17.7% of the leaves developed secondary branches. In the laboratory, dormant buds of *L. japonicum* sprouted constantly 7 days after removal of the main apex (Punetha, 2000). However, under natural conditions most dormant buds of *L. venustum* seemed to lose their viability quickly or never sprouted after the death of the main apex. The leaf life span of even larger plants than in this study may be considerably longer particularly if we consider that the climbing leaves are functionally the same as the shoots of angiosperm lianas (Mueller, 1982a, 1983), but the positive xylem pressure of the roots of up to 66 kPa, which is sufficient to refill embolised tracheids up to 7 m high, may finally limit leaf growth (Ewers *et al.*, 1997). Leaf life span in ferns is quite variable and appears to be negatively correlated with leaf production (Mehltreter and Palacios-Rios, 2003). Longer life spans in fern leaves have been reported only for sterile leaves of *Danaea wendlandii* (39.6 months, Sharpe, 1993) and for the tree ferns *Alsophila salvinii* (24 months, Seiler, 1981, 1995) and *Cibotium taiwanense* (16 months, Chiou 2001). These species were also characterized by a low leaf production of 1.6, 2.5 and 2.3 leaves per year, respectively.

Surprisingly no plant became fertile during the study period. Only twice did I find fertile leaves on plants, these about 500 m from the study site; one grew in the forest and had more than 30 leaves that reached the canopy, the other was only 1.5 m high, but grew in the sun (K. Mehltreter, pers. obs.). For this reason, I suppose that direct sun light is of primary importance for the induction of fertility.

Seasonal leaf growth and leaf production is common in tropical ferns of different habitats (Mehltreter and Palacios-Rios, 2003). Most leaf parameters of *L. venustum* were seasonal and correlated with both mean temperature and precipitation especially after a time lag of one month (Table 1). All leaf parameters were more strongly correlated with precipitation than with temperature, indicating that water is a more limiting factor. In an adjacent area that is consistently wet, a population of the mangrove fern *Acrostichum danaeifolium* also showed seasonal growth patterns, but its leaf growth was more strongly correlated with temperature and there was no time lag between precipitation and leaf growth (Mehltreter and Palacios-Rios, 2003).

Acknowledgments

I thank the staff of the Biological Station of La Mancha for their kind assistance with this study. I am also grateful to Sandra Cardoner, Julien Demenois, José García-Franco, Mónica Palacios-Rios,

Victor Rico-Gray and Javier Tolome for help with fieldwork. Special thanks to Carl Taylor and Jim Montgomery, who revised the manuscript and gave helpful advice. This study was supported by the Instituto de Ecología, A.C. (902-11-796).

Literature Cited

Barrington, D. S. 1993. Ecological and historical factors in fern biogeography. J. Biogeogr. 20: 275–280.

Bullock, S. H. and J. A. Solis-Magallanes. 1990. Phenology of canopy trees of a tropical deciduous forest in Mexico. Biotropica 22:22–35.

Carlquist, S. and E. L. Schneider. 1998. SEM studies on vessels in ferns. X. Osmundaceae and Schizaeaceae. Int. J. Plant Sci. 159:788–797.

Castillo-Campos, G. and M. E. Medina A. 2002. *Árboles y arbustos de la Reserva Natural de La Mancha, Veracruz.* Instituto de Ecología, A.C., Xalapa.

Chiou, W. L. 2001. Phenology of *Cibotium taiwanense* (Dicksoniaceae). Taiwan J. For. Sci. 16: 209–215.

Collinson, M. E. 2002. The ecology of Cainozoic ferns. Rev. Paleobot. Palynol. 119:51–68.

Ewers, F. W., H. Cochard and M. T. Tyree. 1997. A survey of root pressures in vines of a tropical lowland forest. Oecologia 110:191–196.

Foster, R. B. 1996. The seasonal rhythm of fruitfall on Barro Colorado Island. Pp. 151–172, *in* E. G. Leigh, A. S. Rand and D. M. Windsor, eds. *The ecology of a tropical forest: seasonal rhythms and long-term changes, 2nd ed.* Smithsonian Institution Press, Washington, DC.

Geissert, D. and D. Dubreoucq. 1995. Influencia de la geomorfología en la evolución de suelos de dunas costeras en Veracruz, México. Invest. Geogr. UNAM Núm. Esp. 3:37–51.

Kuramatani, M., K. Yagi, T. Murata, M. Tezuka, L. N. Mander, M. Nishiyama and H. Yamane. 2001. Isolation and identification of antheridiogens in the ferns *Lygodium microphyllum* and *Lygodium reticulatum.* Biosci. Biotech. Bioch. 65:2311–2314.

Langeland, K. A. 2002. *Natural area weeds: Old world climbing fern (Lygodium microphyllum).* SS-AGR-21, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Lieberman, D. 1982. Seasonality and phenology in a dry tropical forest in Ghana. J. Ecol. 70: 791–806.

Mehltreter, K. 2002. *Lygodium* und andere Kletterfarne. Palmengarten 66:20–23.

Mehltreter, K. and M. Palacios-Rios. 2003. Phenological studies of *Acrostichum danaeifolium* (Pteridaceae, Pteridophyta) at a mangrove site on the Gulf of Mexico. J. Trop. Ecol. 19: 155–162.

Mendoza, A., B. Perez-García and R. Riba. 1999. Protalic development of *Lygodium heterodoxum* and *Lygodium venustum* (Schizaeaceae). Rev. Biol. Trop. 47:83–92.

Mound, L. A. 2002. *Octothrips lygodii* sp. n. (Thysanoptera: Thripidae) damaging weedy *Lygodium* ferns in southeastern Asia, with notes on other Thripidae reported from ferns. Aust. J. Entomol. 41:216–220.

Mueller, R. J. 1982a. Shoot morphology of the climbing fern *Lygodium* (Schizaeaceae): general organography, leaf initiation, and branching. Bot. Gaz. 143:319–330.

Mueller, R. J. 1982b. Shoot ontogeny and the comparative development of the heteroblastic leaf series in *Lygodium japonicum* (Thunb.) Sw. Bot. Gaz. 143:424–438.

Mueller, R. J. 1983. Indeterminate growth and ramification of the climbing leaves of *Lygodium japonicum* (Schizaeaceae). Amer. J. Bot. 70:682–690.

Nauman, C. E. and D. F. Austin. 1978. Spread of the exotic fern *Lygodium microphyllum* in Florida. Amer. Fern J. 68:65–66.

Punetha, N. 2000. Phenology of the climbing leaf of *Lygodium japonicum* (Thunb.) Sw. (Schizaeaceae). Phytomorphology 50:75–85.

Seiler, R. L. 1981. Leaf turnover rates and natural history of the Central American tree fern *Alsophila salvinii.* Amer. Fern J. 71:75–81.

SEILER, R. L. 1995. Verification of estimated growth rates in the tree fern *Alsophila salvinii*. Amer. Fern J. 85:96–97.

SHARPE, J. M. 1993. Plant growth and demography of the neotropical herbaceous fern *Danaea wendlandii* (Marattiaceae) in a Costa Rican rain forest. Biotropica 25:85–94.

SIGMASTAT 1995. *Sigmastat, statistical software, version 2.0.* Jandel Scientific Software, San Rafael.

TRYON, R. M. and TRYON, A. F. 1982. *Ferns and allied plants with special reference to tropical America.* Springer, New York.

WILLIAMS-LINERA, G. 1999. Leaf dynamics in a tropical cloud forest: phenology, herbivory and life span. Selbyana 20:98–105.

American Fern Journal 96(1):31–44 (2006)

Megalastrum (Dryopteridaceae – Pteridophyta) in Bolivia, with Descriptions of Six New Species

MICHAEL KESSLER
Albrecht-von-Haller-Institut für Pflanzenwissenschaften,
Abteilung Systematische Botanik, Untere Karspüle 2, D-37073 Göttingen, Germany
ALAN R. SMITH
University Herbarium, 1001 Valley Life Sciences Bldg. #2465,
University of California, Berkeley, CA 94720-2465, U.S.A.

ABSTRACT.—A treatment of *Megalastrum* (Dryopteridaceae - Pteridophyta) is provided for Bolivia. We recognize 14 species, of which six are described as new: *M. alticola*, *M. aureisquama*, *M. bolivianum*, *M. ciliatum*, *M. marginatum*, and *M. rupicola*. Also provided are notes and selected specimen citations for the other species, and a key to all Bolivian species of the genus.

Megalastrum (Dryopteridaceae) was described by Holttum (1986) to accommodate species placed by Christensen (1913, 1920) in his informal species group of *Dryopteris subincisa* within *Dryopteris* subg. *Ctenitis*. *Megalastrum* is distinguished from related genera, in particular *Ctenitis*, by venation and indument characters. In *Megalastrum*, the basal basiscopic veinlets of the distal pinnules arise from the penultimate axes (not from the ultimate axes) and the basiscopic lobes of the distal pinnules of 2-pinnate-pinnatifid blades are therefore broadly adnate to the costae. Furthermore, in *Megalastrum* the veins end before the margins in conspicuous clavate tips, as viewed adaxially. On the adaxial axes, *Megalastrum* has coarse, whitish, septate, pointed, antrorsely strigose or spreading hairs, whereas *Ctenitis* and other related genera have fine, usually blunt, reddish, erect to spreading hairs in which the dried cells usually collapse. Transfers of 39 neotropical species previously placed in *Dryopteris* or *Ctenitis* into *Megalastrum* were made by Smith & Moran (1987).

Although *Megalastrum* is nowadays accepted as a distinct genus (e.g., by Tryon and Stolze 1991, Smith and Moran 1995), its species-level taxonomy remains poorly known. The last treatment of the neotropical species now placed in *Megalastrum* was by Christensen (1920), and the genus is in urgent need of monographic treatment (Smith and Moran 1987, 1995). The taxonomy of *Megalastrum* is particularly difficult because herbarium material is usually incomplete due to the large size of many species. During taxonomic work for a forthcoming pteridophyte flora for Bolivia, we have studied 110 gatherings of *Megalastrum* from Bolivia and recognize 14 species, of which six are here described as new. We also provide comments on the remaining Bolivian species of the genus, as well as a key to all Bolivian species. In the case of two widespread species, *M. pulverulentum* and *M. subincisum*, extensive morphological variability leads us to believe that species complexes may be involved. As this situation requires the examination of material from the entire ranges of these taxa, which would have exceeded the scope of this study, we refrain from subdividing or recircumscribing them.

Key to the Bolivian Species of Megalastrum

1. Blades pinnate-pinnatifid; basiscopic pinnules of proximal pinnae sometimes pinnatifid
   2. Costules and veins abaxially with whitish hairs 0.5–1 mm long . . . . . **2. M. bolivianum**
   2. Costules and veins abaxially glabrous or with very few hairs 0.1–0.3 mm long
      3. Rachises densely puberulent; rhizome scales orange to medium brown under magnification and strong light, brown en masse . . . . . **1. M. biseriale**
      3. Rachises glabrous or sparsely puberulent; rhizome scales dark brown
         4. Rachis scales linear, dark brown; sori inframedial . . . . . **3. M. honestum**
         4. Rachis scales broadly lanceolate, pale brown; sori supramedial . . . . . **4. M. yungense**
1. Blades 2-pinnate or more divided
   5. Indusia present
      6. Indusia 0.1–0.3 mm in diameter; blades abaxially with short hairs between the veins . . . . . **5. M. adenopteris**
      6. Indusia 0.8–1 mm in diameter; blades abaxially glabrous or nearly so between the veins . . . . . **14. M. villosulum**
   5. Indusia absent
      7. Scales of costae and costules pale at the bases and darker at the tips, conspicuously dentate . . . . . **10. M. pulverulentum *s.l.***
      7. Scales of costae and costules uniformly colored or slightly darker at the bases, subentire to moderately serrate
         8. Blades adaxially pubescent between and on the veins, the hairs ca. 1 mm long
            9. Rhizome scales golden-brown to pale brown under magnification and strong light, dark brown en masse
               10. Rhizome scales 1–2 mm wide . . . . . **7. M. aureisquama**
               10. Rhizome scales to 1 mm wide . . . . . **12. M. subincisum *s.l.***
            9. Rhizome scales dark brown to blackish
               11. Abaxial blade surfaces densely pubescent; rhizome scales linear-lanceolate . . . . . **8. M. ciliatum**
               11. Abaxial blade surfaces sparsely pubescent; rhizome scales lanceolate . . . . . **11. M. marginatum**
         8. Blades adaxial glabrous or with a few hairs <0.5 mm long on veins and/or surfaces
            12. Short (0.2–0.5 mm long) hairs present abaxially on the veins and/or blade surfaces
               13. Blades densely covered with short hairs of about equal length; long hairs lacking . . . . . **5. M. adenopteris**
               13. Blades with short hairs restricted to abaxial surfaces; long hairs on costules and veins present or lacking
                  14. Costules, costae, and rachises abaxially glabrous or with scattered hairs 0.3–0.6 mm long . . . . . **12. M. subincisum *s.l.***
                  14. Costules, costae, and usually rachises abaxially with dense hairs 0.05–0.2 mm long . . . . . **13. M. vastum**
            12. Short hairs absent abaxially on veins and blade surfaces (may be present on the costae and costules)
               15. Rhizomes and basal petiole scales 1–2 mm wide, pale orange to golden brown under maginification and strong light, brown en masse, entire to finely serrate . . . . . **7. M. aureisquama**
               15. Rhizomes and basal petiole scales to 1 mm wide, orange-brown under maginification and strong light, dark brown en masse, serrate
                  16. Costal and costular scales linear-lanceolate, up to 10 cells wide . . . . . **12. M. subincisum *s.l.***
                  16. Costal and costular cells broadly lanceolate, >10 cells wide
                     17. Blades 3-pinnate-pinnatifid to four-pinnate; 2500–3000 m . . . . . **6. M. alticola**
                     17. Blades 2- to 3-pinnate; 1000–2000 m . . . . . **11. M. rupicola**

Pinnate-pinnatifid species

1. Megalastrum biseriale (Baker) A.R. Sm. & R.C. Moran, Amer. Fern J. 77:127. 1987.

Polypodium biseriale Baker, Syn. fil. 309. 1867. TYPE.—Ecuador. Mt. Tungurahua, *Spruce s.n.* (holotype: K).

This species is recognized by a dense cover of very short hairs on the rachises, and orange to medium brown rhizome scales. It has short, appressed hairs on the costae, a thick laminar texture into which the ultimate veinlets are completely sunken (except for the hydathodes). In contrast to Tryon & Stolze (1991) who state that *M. biseriale* has a thin laminar texture, the Bolivian specimens placed under this name have the thickest laminar texture of any pinnate-pinnatifid species of *Megalastrum* in the country. We have also seen similar, thick-textured specimens from Colombia (*Herrera 9327*, UC) and Ecuador (*Neill 5956*, UC). Different taxa may be involved, but the taxonomy and nomenclature of this species group (including *M. honestum* and *M. yungense*) is complex and requires detailed work. In Bolivia, *M. biseriale* is known from five collections, all made at 1600–2900 m in and close to Cotapata National Park, Nor Yungas, La Paz.

Selected Specimens Examined.—Bolivia. **La Paz:** Prov. Nor Yungas, Estación Biológica de Tunquini, 16°11′S, 67°53′W, 1600 m, 22 Aug 2000, *Eberhardt 204* (LPB, UC); Prov. Nor Yungas, 19.8 km from Yolosa toward Chuspipata, 16°15′S, 67°45′W, 2280 m, 27 Jun 1989, *Fay & Fay 2205* (LPB, MO, UC).

2. Megalastrum bolivianum M. Kessler & A.R. Sm., ***sp. nov.*** TYPE.—Bolivia. **Cochabamba:** Prov. Carrasco, 143 Km antigua carretera Cochabamba-Villa Tunari, 17°07′S, 65°34′W, 1300 m, 23 Aug 1996, *Kessler 7630a* (holotype: UC; isotypes: GOET, LPB). **Fig. 1 D–F.**

Megalastrum laminis pinnato-pinnatifidis, pilis strigosis albescentibus in costis venisque adaxialiter, squamis brunneis latis pilisque longis etiam brevibus in rhachidibus.

Terrestrial; rhizomes compact, scales linear-lanceolate, 5–8 × 0.5–0.8 mm, brown, clathrate, tortuous, remotely denticulate; petioles 10–13 cm × 1.5–2 mm, reddish brown, with dense scales similar to those of the petioles but smaller, and scattered hairs resembling those of the rachises; blades 22–27 × 8.5–10 cm, pinnate-pinnatifid, ovate-lanceolate, broadest at about 2/5 of the length; pinnae short-petiolate, to 5.2 × 1.8 cm, the proximal basiscopic and sometimes acroscopic segments enlarged except on the proximal pinnae; segments 3–4 mm wide, rounded to obtuse; rachises adaxially with dense, mixed indument consisting of reddish, spreading, septate, 5–8-celled hairs 0.8–1.3 mm, 1–2-celled reddish hairs 0.1–0.2 mm, and lanceolate, long-acuminate, brown, clathrate, remotely denticulate scales 1–3.5 × 0.2–0.7 mm, abaxially with similar scales but fewer, whitish hairs 0.5–1 mm; costae and veins adaxially with sparse, whitish, strigose hairs 1–1.5 mm, abaxially with moderately dense scales and hairs resembling those of the rachises abaxially,

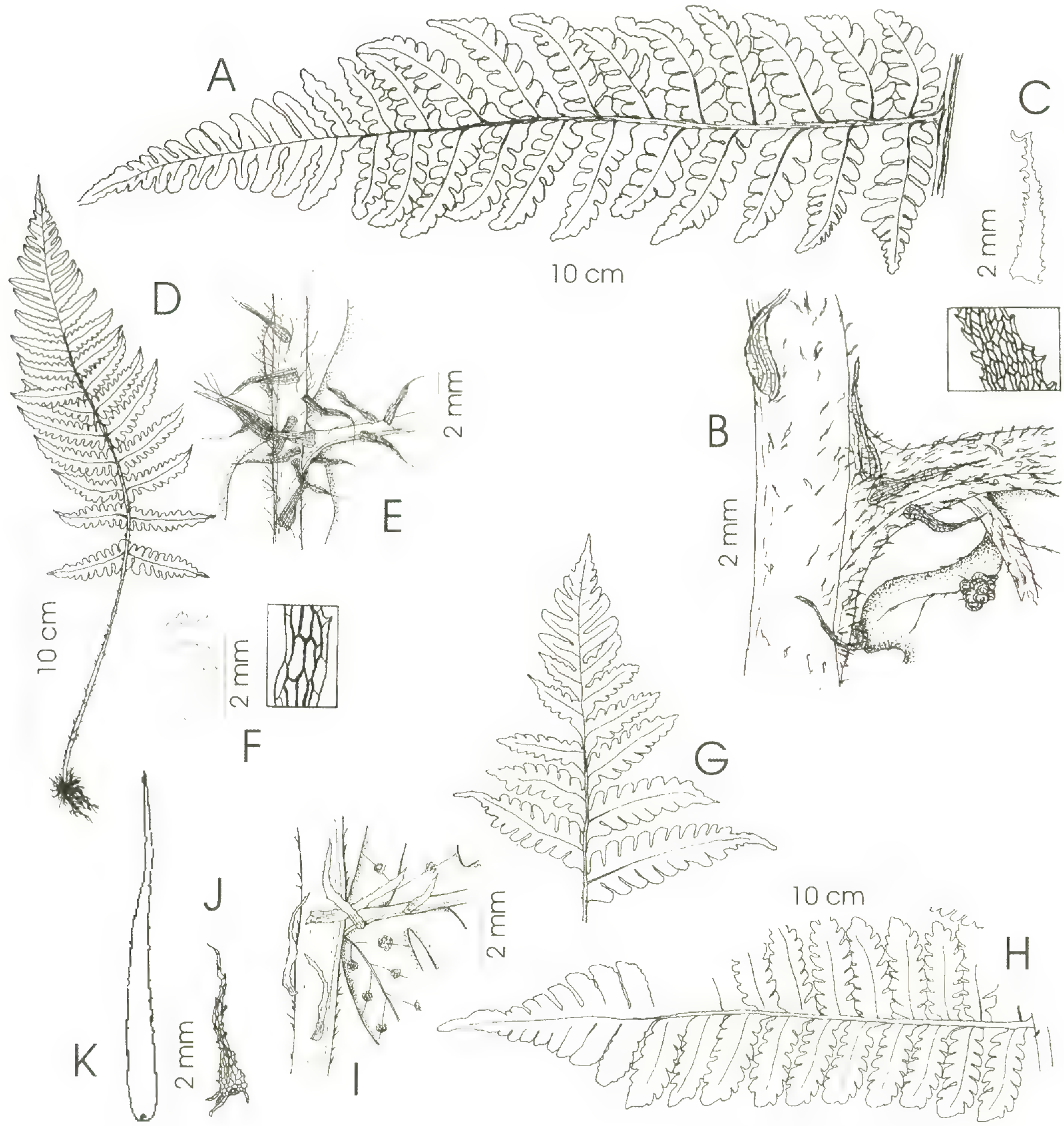


FIG. 1. A.–C. *Megalastrum alticola* M. Kessler & A.R. Sm. A. Proximal pinna. B. Rachis and pinna base. C. Costal scale with cell detail, *Kessler 11945* (UC). D.–F. *Megalastrum bolivianum* M. Kessler & A.R. Sm. D. Leaf. E. Rachis and pinna bases. F. Costal scale with cell detail, *Kessler 7630a* (UC). G.–J. *Megalastrum aureisquama* M. Kessler & A.R. Sm. G. Blade apex. H. Median pinna. I. Rachis and pinna base. J. Costal scale, K. Scale of petiole base, *Kessler* 7379 (UC).

but shorter; laminar tissue thin, adaxially glabrous, abaxially with sparse, appressed, hyaline to orangish hairs 0.2–0.4 mm; veins dark, conspicuous; sori inframedial, 2–4 per segment, distributed along the entire segment, exindusiate.

PARATYPES.—Bolivia. **La Paz:** Prov. Nor Yungas, a 0.5 km de la Estación Biológica Tunquini, subiendo el Río Santa Catalina, 16°11′S, 67°52′W, 1600 m, 23 Aug 1998, *Portugal A. 246* (LPB, UC), *Portugal A. 269* (LPB).

This species is characterized by the long, whitish hairs on the veins of the adaxial blade surfaces, and by the mixture of long and very short hairs and broad, medium brown scales on the rachises. It is a small plant with a thin blades and conspicuous, dark veins. The blade shape is ovate-lanceolate, rather than deltate as in most species of *Megalastrum*. Among the pinnate-pinnatifid species, the only other one with long hairs on the blades is *M. hirsutosetosum* (Hieron.) A.R. Sm. & R.C. Moran from Colombia to Ecuador; however, this species has longer, thinner, denser hairs on both sides of the blades. *Megalastrum bolivianum* is known from only three collections made at two sites in the departments of La Paz and Cochabamba in humid montane forests at 1300–1600 m.

3. Megalastrum honestum (Kunze) A.R. Sm. & R.C. Moran, Amer. Fern J. 77:128. 1987.

Polypodium honestum Kunze, Linnaeae 9:49. 1834. TYPE:—Peru. **Dept. Huánuco:** Pampayacu, *Poeppig 22* (holotype: LZ, destroyed).

Of the pinnate-pinnatifid species, this is relatively large, and can be recognized by lacking hairs on the rachises (or with very few, scattered hairs), dark brown, long, narrow rhizome scales, and inframedial sori. It has thicker petioles than other Bolivian pinnate-pinnatifid congeners (basally to 6 mm thick *vs.* to 3–4 mm in the other species), and the basiscopic pinnule of each pinna is often enlarged (*vs.* not enlarged or enlarged only in the basalmost pinna pair in the other species). The application of the name *M. honestum* to Bolivian material is uncertain, because specimens cited below differ from Peruvian material in several conspicuous characters (larger size, different blade cutting with more pronounced basiscopic pinnules) not mentioned by Christensen (1913) or Tryon & Stolze (1991). Further, contrary to the statement in Tryon & Stolze (1991), the laminar texture of *M. honestum* is thinner than that of *M. biseriale*. We have been unable to locate type material of *M. honestum* (the holotype was destroyed at LZ, and no isotypes are known to us), and the matter may therefore be impossible to settle. Pending a thorough revision of the genus, we tentatively apply the name to specimens cited below. In Bolivia, *M. honestum* is known from eleven collections made in humid foothill forests at 450–1700 m in the departments of Beni, La Paz, Cochabamba, and Santa Cruz.

Selected Specimens Examined.—Bolivia. **Beni:** Prov. Ballivián, 16 km por camino maderero al SW del Km 12 Yucumo-Rurrenabaque, 15°5′S, 67°7′W, 800 m, 03 Aug 1997, *Kessler 10982* (GOET, LPB, UC). **Cochabamba:** Prov. Carrasco, Parque National Carrasco, al S del Campamento Petrolero Ichoa, 17°23′S, 64°30′W, 440 m, 15 Sep 1997, *Acebey 595* (LPB, UC). **La Paz:** Prov. Caranavi, Serranía Bellavista, 46 km Caranavi a Sapecho, 15°39′S, 67°28′W, 1200 m, 30 Aug 1997, *Kessler 11604* (GOET, LPB, UC). **Santa Cruz:** Prov. Ichilo, 4 km SW del Campamento Macuñucú, 17°44′S, 63°35′W, 450 m, 27 Sep 1996, *Kessler 8680* (GOET, LPB, UC).

4. Megalastrum yungense (H. Christ & Rosenst.) A.R. Sm. & R.C. Moran, Amer. Fern J. 77:129. 1987.

Dryopteris yungensis H. Christ & Rosenst., Repert. Spec. Nov. Regni Veg. 5:234. 1908. TYPE:—Bolivia. **La Paz:** Prov. Sud Yungas, Sirupaya, Yanacachi, 2000 m, *Buchtien 493* (holotype: P; isotype: US).

This rare endemic species, known from only two collections, differs from *M. honestum* by its broader, paler rachis scales and supramedial (*vs.* inframedial) sori (Christensen 1920). It is also a smaller species with less pronounced basiscopic pinnules. A specimen from Peru (*Smith 4424*, UC) was placed under this species by Tryon & Stolze (1991), but it has densely hairy rachises and thus falls within our concept of *M. biseriale*.

Specimen Examined.—Bolivia. **Beni:** Prov. Ballivián, 138 km N of Caranavi, 15°12′S, 67°3′W, 700 m, 20 Jun 1989, *Fay & Fay 2051* (LPB, MO).

Decompound species

5. Megalastrum adenopteris (C. Chr.) A.R. Sm. & R.C. Moran, Amer. Fern J. 77:127. 1987.

Dryopteris adenopteris C. Chr., Kongel. Dankse Vidensk. Selsk. Skr., Naturvidensk Math. Afd., ser. 8. 6:85. 1920. Type:—Brazil (South). *Lindman A1313* (holotype: S).

This species, otherwise known from southern Brazil and northern Argentina, is characterized by its short hairs on both blade surfaces; it does not present taxonomic problems in Bolivia. Bolivian specimens have very small indusia that may be hard to see in sori with fully developed sporangia. *Megalastrum adenopteris* is known in Bolivia from three collections (two of which are cited in Smith *et al.* 1999) in semihumid forests at 890–1750 m in Santa Cruz and Tarija departments.

Specimen Examined.—Bolivia. **Tarija:** Prov. A. Arce, P.N. Tariquía, campamento de guardaparques Sidras, senda La Cascada, 22°14′S, 64°32′W, 890 m, 19 Jun 2004, *Jimenez 2468* (GOET, LPB, UC).

6. Megalastrum alticola M. Kessler & A.R. Sm., ***sp. nov.*** TYPE.—Bolivia. **La Paz:** Prov. Nor Yungas, 2 km de Chuspipata hacia Coroico, 16°22′S, 67°49′W, 2900 m, 17 Sep 1997, *Kessler 11945* (holotype: UC; isotypes: GOET, LPB). **Fig. 1 A–C.**

A *Megalastro subinciso* (Willd.) A.R. Sm. & R.C. Moran squamis costarum costularumque latioribus (plus 10 cellulas vs. minus 10 cellulas latis), a *M. rupicola* M. Kessler & A.R. Sm. foliis majoribus subtilioribus dissectis, squamis rhizomatum petiolorumque majoribus differt.

Terrestrial; rhizomes compact, scales linear-lanceolate, 13–25 × 0.5–1 mm, shiny orange-brown, clathrate, erose-denticulate; petioles to 95 cm × 15 mm, basally reddish brown, distally stramineous, shiny when scurf abraded, scales

dense at bases, otherwise petioles covered by a dense scurf of appressed, reduced scales; blades to ca. 120 × 90 cm, 3-pinnate-pinnatifid, deltate, the lowermost pinnae the longest; pinnae to ca. 45 × 20 cm, the proximal basiscopic pinnules enlarged especially on the proximal pinnae; ultimate segments 1.5–3 mm wide, rounded to obtuse; rachises adaxially densely short-hairy along the grooves, otherwise sparsely scaly, the scales 0.2–3 × 0.1–1 mm, brown, subclathrate, erose-denticulate; costae, costules, and veins adaxially with dense, reddish hairs 0.2–0.4 mm, on veins hairs to 0.7 mm long, abaxially with moderately dense, brown, clathrate, ovate-lanceolate, erose-denticulate scales >10 cells wide, 0.3–1.2 × 0.6–1.5 mm, and moderately dense, pale reddish hairs to 0.2–0.5 mm; laminar tissue adaxially glabrous, abaxially with scattered, appressed, reddish hairs 0.2–0.4 mm, margins with regularly spaced, whitish hairs 0.3–0.6 mm; sori inframedial, 1–3 per segment, mostly restricted to segment bases, exindusiate.

PARATYPES.—Bolivia. **Cochabamba:** Prov. Carrasco, Km 108 antiqua carretera Cochabamba a Villa Tunari, 17°9′S, 65°38′W, 2950 m, 22 Jun 1996, *Kessler 6539* (GOET, LPB, UC); same general locality, Km 115, 17°8′S, 65°38′W, 2500 m, 04 Jul 1996, *Kessler 6964* (GOET, LPB, UC); same locality, 2600 m, 05 Jul 1996, *Kessler 7014* (GOET, LPB, UC).

This species differs from *M. subincisum* by its broader costal and costular scales (scales >10 cells wide *vs.* <10 cells wide) and from *M. rupicola* by its larger size with more finely dissected blades, and larger rhizome and petiole scales. It occurs at higher elevations (2500–2950 m) than any other Bolivian *Megalastrum* except *M. aureisquama*. In contrast, *M. subincisum* is found from 250–2250 m. Two of the four known collections have apparently mature, but malformed sporangia, but in the type collection the sporangia and spores appear well-developed. Whether the malformed sporangia are a result of hybridization (but with which parent taxa is unclear, considering that at such high elevations there is only one other, quite different species of the genus) or of environmental stress at the upper elevational limit of the genus is unknown.

7. **Megalastrum aureisquama** M. Kessler & A.R. Sm., ***sp. nov.*** TYPE.—Bolivia. **Cochabamba:** Prov. Carrasco, 137 Km antigua carretera Cochabamba-Villa Tunari, 17°06′S, 65°35′W, 1600 m, *Kessler 7379* (holotype: UC; isotypes: GOET, LPB). **Fig. 1 H–K.**

A *Megalastro subinciso* (Willd.) A.R.Sm. & R.C. Moran squamis rhizomatum petiolorum inferiorisque latissimis (1–2 mm), nitentibus, obscure aureobrunneis, integris usque subtiliter serratis differt.

Terrestrial; rhizomes compact, scales linear-lanceolate, 10–20 × 1–2 mm, shiny, dark golden-brown, subclathrate, entire to finely serrate; petioles to 90+ cm × 18 mm, brown, densely covered with an appressed scurf of dissected scales, bases with dense scales resembling those of the rhizomes but smaller; blades known from incomplete material, at least to 120 × 50 cm, probably deltate, basally 3-pinnate; pinnae short-petiolate, at least to 28 × 15 cm; segments 3–6 mm wide, rounded to obtuse; rachises adaxially with dense, pale

reddish hairs 0.5–1 mm along the grooves, otherwise covered with an appressed scurf of dissected scales and moderately dense, linear-lanceolate, shiny, dark golden-brown, subclathrate, entire to finely serrate scales 5–10 × 0.4–1 mm; costae and veins adaxially with moderately dense, pale reddish hairs 0.4–0.7 mm, abaxially with moderately dense, appressed, linear-lanceolate, shiny, dark golden-brown, subclathrate, entire to finely serrate scales 2–6 × 0.2–0.7 mm; laminar tissue glabrous on both sides or adaxially with sparse, strigose, whitish hairs 0.5–1 mm; sori supramedial, 4–8 per segment, distributed along the entire segment, exindusiate.

PARATYPES.—Bolivia. **Cochabamba:** Prov. Carrasco, Km 113 antiqua carretera Cochabamba a Villa Tunari, 17°7′S, 65°38′W, 2600 m, 03 Jul 1996, *Kessler 6908* (GOET, LPB, UC); same general locality, Km 116, 17°8′S, 65°38′W, 2400 m, 06 Jul 1996, *Kessler 7033* (GOET, LPB, UC); same general locality, Km 130, 17°7′S, 65°36′W, 2000 m, 11 Jul 1996, *Kessler 7185* (GOET, LPB, UC); same general locality, Km 100, 17°11′S, 65°38′W, 3200 m, 20 Aug 1996, *Kessler 7522* (GOET, LPB, UC); same general locality, Km 143, 17°7′S, 65°34′W, 1300 m, 23 Aug 1996, *Kessler 7644* (GOET, LPB, UC); same general locality, Km 134, 17°7′S, 65°34′W, 1650 m, 26 Aug 1996, *Kessler 7789* (GOET, LPB, UC).

This species is readily recognized by its very broad, shiny, dark golden-brown, entire to finely serrate rhizome and basal petiole scales. Similar, but smaller scales are found on the rachises, costae, and costules. It is probably most closely related to *M. subincisum*, but that species has much narrower, darker, and more strongly denticulate rhizome and petiole scales. In *M. aureisquama*, most specimens found below 2000 m are glabrous on the adaxial blade surfaces, whereas most high-elevation specimens have long hairs on the costules and veins. *Megalastrum aureisquama* is fairly common at 1300–3200 m in humid montane forests in the very wet Chapare region of Dpto. Cochabamba.

8. Megalastrum ciliatum M. Kessler & A.R. Sm., ***sp. nov.*** TYPE.—Bolivia. **La Paz:** Prov. Sud Yungas, camino Chulumani-Ocobaya, 2.5 km después de Chulumani, 1600 m, *Schmit 422* (holotype UC; isotype LPB). **Fig. 2 A–C.**

Megalastrum foliis tripinnato-pinnatifidis, pilis longis adaxialiter in venis, abaxialiter etiam inter venas instructis, squamis costalibus denticulatis, iisdem rhizomatum subtiliter ciliatis notatum.

Terrestrial; rhizomes compact, scales linear-lanceolate, 8–14 × 1–2 mm, shiny, dark brown, not clathrate, short-ciliate; petioles 33 cm × 4 mm, stramineous, shiny, scales basally dense, distally scattered, appressed and reduced in size; blades known only from incomplete material, 45+ × ca. 40 cm, probably deltate, basally 3-pinnate, the lowermost or the 2nd pinna pair the longest; pinnae short-petiolate, to 23 × 12 cm, the proximal basiscopic segments enlarged especially on the proximal pinnae; segments 3–5 mm wide, rounded to obtuse; rachises adaxially densely hairy along the grooves, the hairs pale orangish, 0.6–1.2 mm long, otherwise sparsely hairy and scaly, the scales 1–3 × 0.1–0.4 mm, dark brown, not clathrate, denticulate; costae and veins

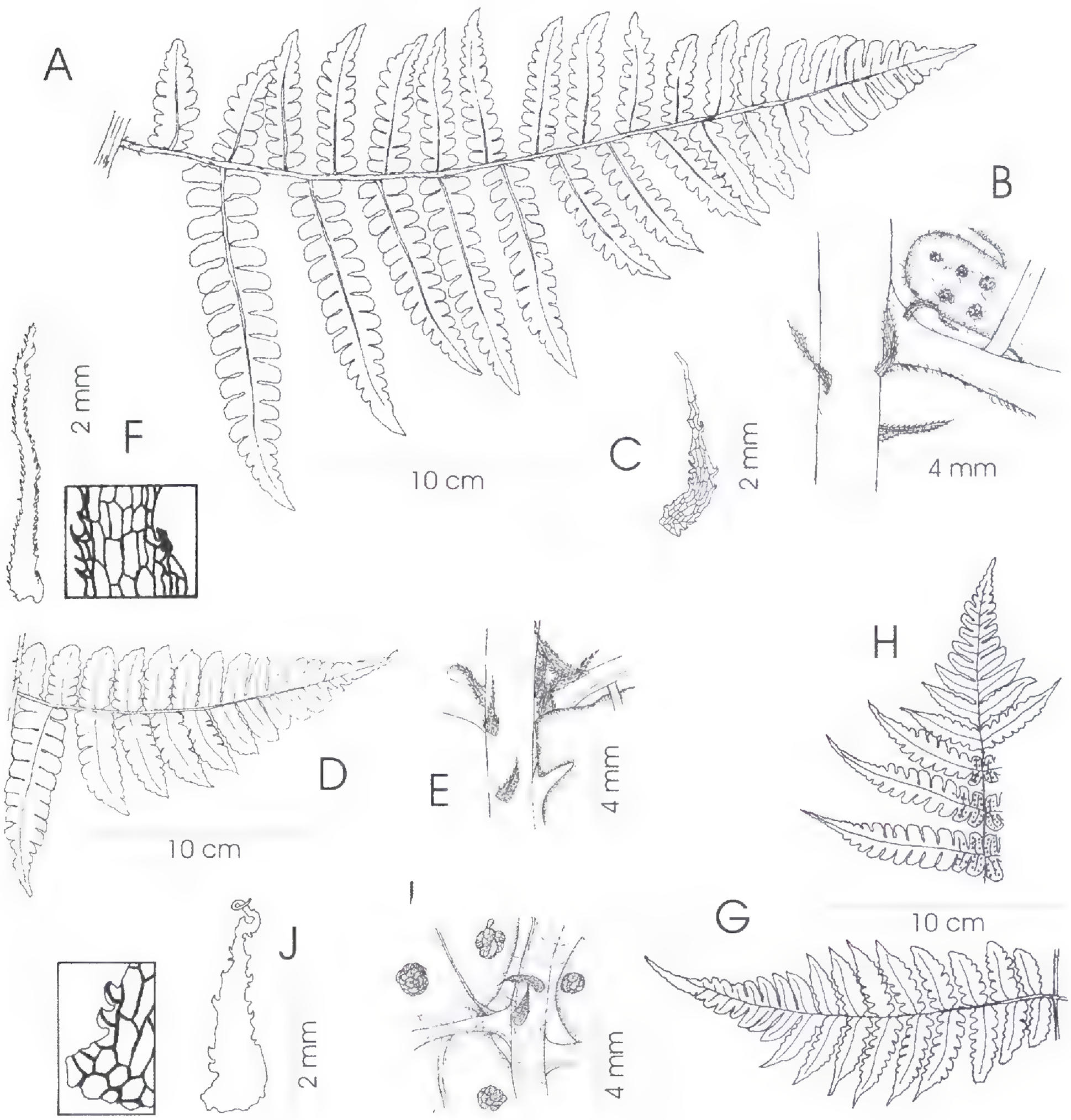


FIG. 2. A.–C. *Megalastrum ciliatum* M. Kessler & A.R. Sm. A. Proximal pinna. B. Rachis and pinna base. C. Costal scale, *Schmit 422* (UC). D.–F. *Megalastrum rupicola* M. Kessler & A.R. Sm. D. Proximal pinna. E. Rachis and pinna bases. F. Costal scale with cell detail, *Quintana 41* (UC). G.–J. *Megalastrum marginatum* M. Kessler & A.R. Sm. G. Blade apex. H. Median pinna. I. Rachis and pinna base. J. Costal scale with cell detail, *Portugal 538* (UC).

adaxially strigose with moderately dense, whitish hairs 0.5–0.8 mm, abaxially with moderately dense, lax, spreading, whitish hairs 0.5–0.8 mm, and with scattered scales resembling those of the rachises but smaller; laminar tissue adaxially glabrous, abaxially moderately hairy with hairs similar to those of the veins; sori medial, 4–6 per segment, distributed along the entire segment, exindusiate.

This species is characterized by 3-pinnate leaves with long hairs on the blades adaxially and abaxially (both on and between the veins), denticulate, concolorous costal scales, and dark brown, finely ciliate rhizome scales. It may

be closest to *M. pulverulentum*, but that species has strongly bicolorous costal scales with much coarser teeth. *Megalastrum ciliatum*, named for its short-ciliate rhizome scales, is known only from the type collection in secondary humid montane forests near the town of Chulumani on a semi-isolated mountain range in the Bolivian Yungas. This range supports a number of locally endemic plant taxa (S.G. Beck, pers. comm.), and the present species may also be restricted to this area.

9. Megalastrum marginatum M. Kessler & A.R. Sm., ***sp. nov.*** TYPE.—Bolivia. **La Paz:** Prov. Nor Yungas, Cerro Hornuni, 16°01′S, 67°52′W, 1850 m, 27 Nov 1998, *Portugal 538* (holotype: UC; isotype: LPB). **Fig. 2 D–F.**

A *Megalastro rupicola* M. Kessler & A.R.Sm. in foliis basaliter tripinnatis, pilis longis costis venisque ambarum paginarum laminarum, squamis rhizomatum brevioribus fuscatioribusque, soris supramedialis positis differt.

Terrestrial; rhizomes compact, scales linear-lanceolate, 5–10 × 0.2–0.6 mm, shiny brown, subclathrate, remotely denticulate; petioles 30 cm × 3 mm, basally brown, distally stramineous, shiny, scales basally dense, distally scattered and reduced in size; blades 50 × 35 cm, deltate, basally 3-pinnate, the lowermost pinnae the longest; pinnae sessile, to 17 × 13 cm, the proximal basiscopic segments enlarged, especially on the proximal pinnae; segments 3–5 mm wide, obtuse; rachises adaxially densely hairy along the grooves, otherwise sparsely scaly, the scales 1–3 × 0.1–0.8 mm, brown, clathrate, denticulate, sometimes with a few admixed hairs; costae and veins adaxially with moderately dense, whitish hairs 1–1.5 mm, abaxially with similar but somewhat sparser and shorter hairs and with scattered scales resembling those of the rachises but smaller; laminar tissue glabrous on both sides; sori supramedial, 2–8 per segment, distributed along the entire segment, exindusiate.

PARATYPE.—Bolivia. **La Paz:** Prov. Sud Yungas, camino Chulumani-Ocobaya, 2.5 km después de Chulumani, 16°25′S, 67°31′W, 1600 m, 01 Jun 1992, *Schmit 440* (LPB).

This species is most similar to *M. rupicola*, with which it grows, but differs by its basally 3-pinnate blades, long hairs on the costae and veins on both sides of the blades, shorter and darker rhizome scales, and supramedial sori. *Megalastrum marginatum* is known from only two collections made in humid montane forests (the type was found along a creek) at 1600–1850 m.

10. Megalastrum pulverulentum (Poir. in Lam.) A.R. Sm. & R.C. Moran, *s.l.*, Amer. Fern J. 77:129. 1987.

Polypodium pulverulentum Poir. in Lam., Encycl. Méth. 5:555. 1804. TYPE.—*Plumier*, Traité foug. Amér. *t. 34*, 1705, based on a plant from Hispaniola.

On average, Bolivian specimens of this widespread species have shorter rachis and petiole scales than specimens from northern South America and Mesoamerica (although the extremes overlap). Only one specimen (*Kessler*

9088, LPB, GOET, UC) has scales approaching those of typical, northern *M. pulverulentum*. Within Bolivia, two morphologically and geographically distinct populations can be distinguished. One (morph 1) occurs in south-central Bolivia and bears long hairs on the adaxial leaf surfaces. The other (morph 2) is found in central Bolivia and has almost glabrous adaxial and abaxial leaf surfaces, as well as slightly broader scales on the rhizomes and petiole bases. These forms may warrant treatment as distinct taxa, but detailed studies throughout the range of the species (or species complex) is needed to adequately evaluate its morphological variability. For example, some specimens of morph 2 closely resemble *M. pulverulentum* var. *heydei* (C. Chr.) A.R. Sm. & R.C. Moran of Mesoamerica, but the similarity is probably superficial. In Bolivia, *M. pulverulentum* is known from 23 collections made in humid forests at (400)1000–2900 m in the departments of Chuquisaca, Cochabamba, and Santa Cruz.

SELECTED SPECIMENS EXAMINED.—Bolivia. **Chuquisaca:** Prov. Calvo, Ñahuañanca, 19°18′S, 63°44′W, 1061 m, 08 Jul 2001, *Huaylla 211* (LPB) (morph 1). **Cochabamba:** Prov. Carrasco, Km 130 antiqua carretera Cochabamba a Villa Tunari 17°7′S, 65°36′W, 2000 m, 11 Jul 1996, *Kessler 7188* (GOET, LPB, UC) (morph 2); Prov. Carrasco, 10 km aproximadamente desde el campamento Israel en dirección al Río Sacta, entrando a la confluencia del límite del Parque Nal. Carrasco y el Río Zabala, 17°17′S, 64°52′W, 400 m, 19 Jan 2000, *Jimenez 224* (AAU, LPB) (morph 2). **Santa Cruz:** Prov. Valle Grande, Loma Larga, 1 km a Masicurí, 18°46′S, 63°54′W, 1900 m, 29 May 1996, *Kessler 6171* (LPB, UC) (morph 1); Prov. Florida, Parque Nacional Amboró, 7 km NNE by air from Mairana, 18°3′S, 65°55′W, 2000 m, 23 Jul 1994, *Moran 5911* (UC) (morph 1).

11. Megalastrum rupicola M. Kessler & A.R. Sm., ***sp. nov.*** TYPE.—Bolivia. **La Paz:** Prov. Nor Yungas, Estación Biológica de Tunquini, Bajo Hornuni, senda del campo de Don Pedro al camino de la mina, 16°12′S, 67°53′W, *Quintana 41* (holotype: UC; isotypes: GOET, LPB). **Fig. 6.**

A *Megalastro subinciso* (Willd.) A.R. Sm. & R.C. Moran squamis costarum costularumque valde latioribus, pallidioribus, translucentioribus differt; etiam ad staturam minorem segmentos ultimosque obtusiores quam *M. subincisum* tendens.

Terrestrial; rhizomes compact, scales linear-lanceolate, 8–17 × 0.3–0.8 mm, shiny orange-brown, clathrate, remotely denticulate; petioles 30–60 cm × 3–8 mm, basally rich brown, distally stramineous, shiny, scales basally dense, distally scattered and reduced in size; blades 40–80 × 30–50 cm, 2-pinnate-pinnatifid, deltate, the lowermost pinnae the longest; pinnae short-petiolate, to 15 × 8.5 cm, the proximal basiscopic segments enlarged especially on the proximal pinnae; segments 2.5–5 mm wide, rounded to obtuse; rachises adaxially densely short-hairy along the grooves, otherwise sparsely scaly, the scales 0.2–4.5 × 0.1–0.8 mm, brown, clathrate, denticulate, sometimes with a few admixed hairs; costae and veins adaxially with dense, reddish hairs 1–1.5 mm, abaxially with scattered brown, clathrate, denticulate scales

0.2–3 × 0.1–0.8 mm, and scattered whitish, spreading hairs to 1 mm; laminar tissue glabrous on both sides or with very few hairs to 1 mm; sori medial, 2–10 per segment, distributed along the entire segment, exindusiate.

PARATYPES.—Bolivia. **Cochabamba:** Prov. Chapare, Territorio Indígena Parque Nacional Isiboro-Sécure, Cordillera de Mosetenes, arriba de la Laguna Carachupa, 16°14′S, 66°25′W, 1350 m, 30 Aug 2003, *Kessler 13070* (LPB, UC). **La Paz:** Prov. B. Saavedra, Pauji-Yuyo, entre Apolo y Charazani, 15°2′S, 68°29′W, 1050 m, 08 Jun 1997, *Kessler 9900* (GOET, LPB, UC); Prov. Nor Yungas, Estación Biológica de Tunquini, Hornuni Bajo, senda del campo de Don Pedro al pajonal atrás del Río Cedroni, 16°42′S, 67°52′W, 1900 m, 24 Jul 2001, *Bach 1234* (GOET, LPB, UC); Estación Biológica de Tunquini, 16°11′S, 67°53′W, 1600 m, 18 Aug 2000, *Eberhardt 142* (LPB, NY, UC); same locality, 1690 m, 22 Aug 1999, *Portugal 223* (LPB, UC), 1680 m, 10 Mar 1998, *Portugal 366* (LPB, UC), 1680 m, 10 Mar 1998, *Portugal 368* (LPB), 1680 m, 10 Mar 1998, *Portugal 370* (LPB, UC), 1760 m, 10 Apr 1998, *Portugal 401* (LPB, UC), 1790 m, 10 Jul 1998, *Portugal 452* (LPB, UC).

This species differs from *M. subincisum* by its broader, paler, more translucent costal and costular scales. It also tends to be smaller and to have blunter ultimate segments than *M. subincisum*. The proximal pinnae of *M. rupicola* typically are slightly falcate. We include here specimens reported as *M. connexum* (Kaulf.) A.R. Sm. & R.C. Moran *vel aff.* by Smith *et al.* (1999). However, material of *M. connexum* from southeastern Brazil and Paraguay has much fewer, paler, less spreading, and laxer scales. One collection (*Eberhardt 402*, UC) is morphologically intermediate between *M. rupicola* and *M. subincisum* and has malformed sporangia; it may represent a hybrid. *Megalastrum rupicola* is a locally common species at 1050–1900 m in humid montane forests, especially along streams.

12. Megalastrum subincisum (Willd.) A.R. Sm. & R.C. Moran, *s.l.*, Amer. Fern J. 77:129. 1987.

Polypodium subincisum Willd., L. Sp. Pl. ed. 4, 5:202. 1810. TYPE.—Venezuela. Caracas, *Bredemeyer s.n.* (holotype: B, *Herb. Willd. 19701*; photos F, US).

Throughout its extensive range (Mexico and West Indies to Bolivia) this species varies considerably in size, blade dissection, and length and density of hairs on the blades; Further studies may reveal it to be a species complex. Usually, the leaves are glabrous except for dense, short hairs on the costae and costules on both leaf surfaces and for scattered short hairs on the segment margins. However, several Bolivian specimens clearly stand apart: one morph (*Kessler 10897, 11131*) has scattered, very short, erect hairs on the abaxial leaf surfaces between the veins, whereas yet another form (*Acebey 730, Krömer 100*) has long hairs on the costules and veins of the adaxial leaf surfaces. In Bolivia, it is known from 24 collections in humid forests at 250–2250 m in the departments of Beni, Cochabamba, La Paz, and Santa Cruz.

SELECTED SPECIMENS EXAMINED.—Bolivia. **Beni:** Prov. Ballivián, Km 16 por camino maderero al SW del Km 12 Yucumo-Rurrenabaque, 15°5′S, 67°7′W, 700 m, 31 Jul 1997, *Kessler 10897* (GOET, LPB, UC); Puente Río Quiquibey 4 kms hacia San Borja, 700 m, 14 Jul 1979, *Beck 1705* (LPB, UC). **Cochabamba:** Prov. Carrasco, Parque National Carrasco, al S del Campamento Petrolero Ichoa, 17°23′S, 64°23′W, 660 m, 21 Sep 1997, *Acebey 730* (LPB, UC); Prov. Ayopaya, Comunidad Pampa Grande, subiendo por el lecho del primer afluente que uno encuentra al cruzar y subir por el Río Pampa Grande, 16°40′S, 66°28′W, 2260 m, 15 Sep 2002, *Jimenez 1641* (LPB, UC). **La Paz:** Prov. Iturralde, Río San Antonio, 46 km de Ixiamas a Alto Madidi, 13°38′S, 68°26′W, 300 m, 13 Aug 1997, *Kessler 11131* (GOET, LPB, UC); Prov. Sud Yungas, Alto Beni, Sapecho, Colonia Tupiza, 15°32′S, 67°18′W, 735 m, 29 Oct 1997, *Krömer 100* (GOET, LPB, UC); Prov. Caranavi, Serranía Bellavista, 46 km Caranavi a Sapecho, 15°39′S, 67°28′W, 1200 m, 30 Aug1997, *Kessler 11621* (GOET, LPB, UC). **Santa Cruz:** Prov. Ñuflo de Chávez, Serranía San Lorenzo, 2 km E of Estancia Las Maras, 16°15′S, 62°35′W, 700 m, 03 Nov 1985, *Killeen 1383* (LPB, UC).

13. Megalastrum vastum (Kunze) A.R. Sm. & R.C. Moran, Amer. Fern J. 77:129. 1987.

Polypodium vastum Kunze, Linnaea 9:50. 1834. TYPE.—Peru. **Dpto. Huánuco:** inter Pampayacu et Cocheros et ad Ventanilla de Cassapi, *Poeppig 217* (holotype: B; isotypes: B, L; photos F, US of L).

This species is closely related to *M. subincisum*, but differs by its dense, regularly spaced, 0.05–0.2 mm long hairs on the costae, costules, and usually rachises abaxially. Given the variability of *M. subincisum*, some specimens of that species approach *M. vastum*, and the distinction between these species is not always clear. *Megalastrum vastum* is a rare species in Bolivia, being known from only three collections made in humid forests at 1100–1300 m in the departments of Cochabamba and La Paz. We have not examined two collections cited by Christensen (1920): La Paz: Prov. Nor Yungas, Polo-Polo prope Coroico, 1100 m, 1912, *Buchtien 3588* (R), *3609* (R).

SELECTED SPECIMEN EXAMINED.—Bolivia. **Cochabamba:** Prov. Chapare, Territorio Indígena Parque Nacional Isiboro-Sécure, Cordillera de Mosetenes, Laguna Carachupa, 16°14′S, 66°25′W, 1300 m, 29 Aug 2003, *Kessler 12995* (LPB, UC).

14. Megalastrum villosulum (C. Chr.) A.R. Sm. & R.C. Moran, Amer. Fern J. 77:129. 1987.

Dryopetris villosula C. Chr., Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk Math. Afd., ser. 8. 6:89. 1920. TYPE.—Bolivia. **La Paz:** Prov. Nor Yungas, Uchumachi, *Bang 2394* (holotype: B; isotypes: NY, US).

This endemic and distinctive species is the only persistently and conspisuously indusiate *Megalastrum* in Bolivia. It is probably closely related to *M. andicola* (C. Chr.) A.R. Sm. & R.C. Moran from Colombia, Ecuador, and Peru, which may be expected also to occur in Bolivia. It differs from *M. villosulum* by lacking small, appressed, glandular hairs on the abaxial blade surfaces and instead bearing dense, long hairs. Until a few years ago, *M. villosulum* was known only from the type collection, but we now have seen five gatherings from humid montane forests at 1800–2150 m in the departments of La Paz, Cochabamba, and Santa Cruz.

Selected Specimens Examined.—Bolivia. **Cochabamba:** Prov. Ayopaya, Comunidad Pampa Grande, subiendo por el sendero río arriba, pasando la primera área de cultivo, 16°40′S, 66°28′W, 2150 m, 09 Jul 2002, *Jimenez 1428* (LPB, UC). **La Paz:** Prov. F. Tamayo, Parque Nacional Madidi, Quebrada Jatun Chiriuno, 31 km en linea recta al E de Apolo por el camino a San José, 14°30′S, 68°14′W, 1850 m, 19 Jul 2002, *Fuentes 5131* (LPB, UC). **Santa Cruz:** Prov. Valle Grande, Loma Larga, 5 km a Vallegrande, 18°43′S, 63°54′W, 2100 m, 08 Jun 1996, *Kessler 6379* (LPB, UC).

Acknowledgments

We thank the curators at B, NY, P, and US for enabling us to study the relevant type specimens under their care, the Dirección Nacional de Conservación de la Biodiversidad and the Herbario Nacional de Bolivia for supporting our research, Marcus Lehnert for translating the Latin diagnoses, and Anika Richter for preparing the drawings.

Literature Cited

Christensen, C. 1920. A monograph of the genus *Dryopteris*, part I. The tropical American pinnatifid-bipinnatifid species. Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Math. Afd., ser. 7. 10:55–282.

Christensen, C. 1920. A monograph of the genus *Dryopteris*, part II. The tropical American bipinnate-decompound species. Kongel. Danske Vidensk. Selsk. Skr., Naturvidensk. Math. Afd., ser. 8. 6:3–132.

Holttum, R. E. 1986. Studies in the fern genera related to *Tectaria* Cav. VI. A conspectus of genera in the Old World regarded as related to *Tectaria*, with descriptions of two genera. Gard. Bull. Straits Settlem. 39:153–167.

Smith, A. R., M. Kessler and J. Gonzales. 1999. New records of pteridophytes from Bolivia. Amer. Fern J. 89:244–266.

Smith, A. R. and R. C. Moran. 1987. New combinations in *Megalastrum* (Dryopteridaceae). Amer. Fern J. 77:124–130.

Smith, A. R. and R. C. Moran. 1995. *Megalastrum*. Pages 202–204. In: R.C. Moran & R. Riba (eds.). *Flora Mesoamericana, Vol. 1, Psilotaceae a Salviniaceae.* Univ. Nacional Autónoma de México, Mexico City.

Tryon, R. M. and R. G. Stolze. 1991. Pteridophyta of Peru, Part IV, 17, Dryopteridaceae. Fieldiana, Bot. n.s. 27:1–176.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerox copies acceptable), including review copies of illustrations and originals of illustrations. After review, submission of final versions of manuscripts on diskette (in PC- or Mac-compatible formats) is strongly encouraged. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, short, informative abstract, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Do not submit right-justified copy, avoid footnotes, and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence et al., 1968, Hunt Botanical Library, Pittsburgh) and its supplement (1991). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, omit the abstract and put all references parenthetically in text. Use *Index Herbariorum* (Regnum Veg. 120:1–693. 1990) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matter of form or style, consult recent issues of *American Fern Journal* and *The Chicago Manual of Style*, 14th ed. (1993, Univ. Chicago Press, Chicago). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

PTERIDOLOGIA ISSUES IN PRINT

The following issues of *Pteridologia*, the memoir series of the American Fern Society, are available for purchase:

1. Wagner, David H. 1979. Systematics of Polystichum in Western North America North of Mexico. 64 pp. $10.00 postpaid.

2A. Lellinger, David B. 1989. The Ferns and Fern-allies of Costa Rica, Panama, and the Chocó (Part 1: Psilotaceae through Dicksoniaceae). 364 pp. $32.00 postpaid.

3. Lellinger, David B. 2002. A Modern Multilingual Glossary for Taxonomic Pteridology. 263 pp. $28.00 postpaid.

Send your order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

AMERICAN FERN JOURNAL ON MICROFICHE

Volumes 1–61 of the *American Fern Journal* are available as archival quality, silver positive microfiches. Single volumes or the entire run may be purchased. The fiches are easily read with 10x or greater magnification (using a dissecting microscope and transmitted illumination or a fiche reader). Silver negative microfiches of vols. 1–50 are also available. The price is $4.00 per volume or $244.00 per set of 61 volumes, postpaid.

Send your inquiry or order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

FIDDLEHEAD FORUM

The editor of the Bulletin of the American Fern Society welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

SPORE EXCHANGE

Ms. Denia Mandt, 12616 Ibbetson Ave., Downey, CA 90242-5050, is Director. Spores exchanged and lists of available spores sent on request. http://amerfernsoc.org/sporexy.html

GIFTS AND BEQUESTS

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Back issues of the Journal and cash or other gifts are always welcomed and are tax-deductible. Inquiries should be addressed to the Secretary.

VISIT THE AMERICAN FERN SOCIETY'S WORLD WIDE WEB HOMEPAGE: http://www.amerfernsoc.org/

AMERICAN FERN JOURNAL

Volume 96

Number 2

April–June 2006

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

On the Phylogenetic Position of *Cystodium*: It's Not a Tree Fern – It's a Polypod
Petra Korall, David S. Conant, Harald Schneider, Kunihiko Ueda, Harufumi Nishida and *Kathleen M. Pryer* **45**

Gametophytes of Four Tropical, Terrestrial *Huperzia* Species (Lycopodiaceae)
Dean P. Whittier **54**

SHORTER NOTES

The New Flavone Ester Apigenin 7-*O*-*p*-hydroxybenzoate and Three Di-*C*-Glycosylflavones from *Pteris vittata*
Filippo Imperato **62**

REVIEW

Flora de la República de Cuba: Serie A. Plantas Vasculares. Fascículo 11
Robbin C. Moran **66**

The American Fern Society

Council for 2006

TOM RANKER, University Museum, Campus Box 265, University of Colorado, Boulder, CO 80309-0265.
President

DAVID S. CONANT, Dept. of Natural Sciences, Lyndon State College, Lyndonville, VT 05851.
President-Elect

W. CARL TAYLOR, 800 W. Wells St., Milwaukee Public Museum, Milwaukee, WI 53233-1478.
Secretary

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916-1110.
Treasurer

GEORGE YATSKIEVYCH, Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299.
Membership Secretary

JAMES D. MONTGOMERY, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.
Curator of Publications

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Journal Editor

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Memoir Editor

ROBIN HALLEY, 1418 Park Row, La Jolla, CA 92037-3710
DAVID SCHWARTZ, 9715 Christey Way, Bakersfield, CA 93312-5617
Bulletin Editors

American Fern Journal

EDITOR

R. JAMES HICKEY ..... Botany Department, Miami University, Oxford, OH 45056,
ph. (513) 529-6000, e-mail: hickeyrj@muohio.edu

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47405-6801
GARY K. GREER ..... Biology Dept., Grand Valley State University, Allendale, MI 49401
CHRISTOPHER H. HAUFLER ..... Dept. of Ecology and Evolutionary Biology, University of Kansas,
Lawrence, KS 66045-2106
ROBBIN C. MORAN ..... New York Botanical Garden, Bronx, NY 10458-5126
JAMES H. PECK ..... Dept. of Biology, University of Arkansas–Little Rock,
2801 S. University Ave., Little Rock, AR 72204

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at The American Fern Society, % Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299. Periodicals postage paid at St. Louis, MO, and additional entry.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.

Changes of address, dues, and applications for membership should be sent to the Membership Secretary.

General inquiries concerning ferns should be addressed to the Secretary.

Back volumes are avalible for most years as printed issues or on microfiche. Please contact the Back Issues Curator for prices and availability.

Subscriptions:

Society Membership – USA, Canada, Mexico (includes Journal and Fiddlehead Forum)	$25
Society Membership – All other countries (includes Journal and Fiddlehead Forum)	$32
Society Life Membership – $300 (add $140 mailing surcharge for outside USA, Canada, Mexico)	
Regular Membership – USA, Canada, Mexico (includes Fiddlehead Forum)	$12
Regular Membership – All other countries (includes Fiddlehead Forum)	$19
Institutional Membership – $35 to USA, Canada, Mexico; $45 elsewhere (-$2 agency fee)	

POSTMASTER: Send address changes to American Fern Journal, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

American Fern Journal 96(2):45–53 (2006)

On the Phylogenetic Position of *Cystodium*: It's Not a Tree Fern – It's a Polypod!

PETRA KORALL[1]
Department of Biology, Duke University, Durham, NC 27708, USA,
and Swedish Museum of Natural History, Stockholm, Sweden

DAVID S. CONANT
Natural Science Department, Lyndon State College, Lyndonville, VT 05851, USA

HARALD SCHNEIDER
Albrecht-von-Haller Institute of Plant Sciences,
Georg-August University, Göttingen, Germany

KUNIHIKO UEDA
Division of Life Science, Graduate School of Natural Science and Technology,
Kanazawa University, Kanazawa, Japan

HARUFUMI NISHIDA
Faculty of Science and Engineering, Chuo University, 1-13-27 Kasuga,
Bunkyo, Tokyo 112-8551, Japan

KATHLEEN M. PRYER
Department of Biology, Duke University, Durham, NC 27708, USA

ABSTRACT.—The phylogenetic position of *Cystodium* J. Sm. is studied here for the first time using DNA sequence data. Based on a broad sampling of leptosporangiate ferns and two plastid genes (*rbcL* and *atpB*), we show that *Cystodium* does not belong to the tree fern family Dicksoniaceae, as previously thought. Our results strongly support including *Cystodium* within the large polypod clade, and suggest its close relationship to the species-poor grade taxa at the base of the polypod topology (*Sphenomeris* and *Lonchitis*, or *Saccoloma* in this study). Further studies, with an expanded taxon sampling within polypods, are needed to fully understand the more precise phylogenetic relationships of *Cystodium*.

Cystodium J. Sm., with its single species *C. sorbifolium* (Sm.) J. Sm., has traditionally been included in Dicksoniaceae (Christensen, 1938; Pichi Sermolli, 1977; Tryon and Tryon, 1982; Kramer, 1990; Stevenson and Loconte, 1996) and, more specifically, has been considered to be closely related to *Dicksonia* L'Hér. (Hooker, 1844; Copeland, 1947; Holttum and Sen, 1961). *Cystodium* grows in lowland rainforests from Borneo to New Guinea and adjacent islands such as the Bismarck and Louisiade Archipelagos, and the Solomon Islands (Holttum, 1963; Croft, 1986; Kramer, 1990). It has a creeping, hairy, dictyostelic rhizome with large, bipinnate leaves that are two meters or more in length (Croft, 1986). The terminal sori are covered by a true indusium

[1] Corresponding author. Current address: Department of Phanerogamic Botany, Swedish Museum of Natural History, Box 50007, SE-104 05 Stockholm, SWEDEN. E-mail: petra.korall@nrm.se.

and an adaxial, false indusium, a modified part of the leaf (Croft, 1986). This indusium type has been highlighted as an important feature that unites *Cystodium* with *Dicksonia*, *Thyrsopteris* Kunze, *Culcita* C. Presl, *Calochlaena* (Maxon) Turner & White, and *Cibotium* Kaulf. within Dicksoniaceae (Kramer, 1990).

Cystodium was not included in any of the large-scale molecular phylogenetic studies in the last decade that focused on the relationships of monilophytes and leptosporangiate ferns (Hasebe *et al.*, 1994, 1995; Pryer *et al.*, 1995; Rothwell, 1999; Pryer *et al.*, 2001, 2004; Schneider *et al.*, 2004; Wikström and Pryer, 2005). In several of these studies, Dicksoniaceae, represented by one or more of its genera (never *Cystodium*), was consistently found to be closely related to Cyatheaceae in a clade termed the "tree ferns", where most of the taxa are arborescent. When more than one representative of Dicksoniaceae was included in these DNA studies, the monophyly of the family was often called into question (Hasebe *et al.*, 1994, 1995; Wolf *et al.*, 1999; Pryer *et al.*, 2004). In a strictly morphological cladistic analysis by Stevenson and Loconte (1996), where *Cystodium* was included along with other dicksoniaceous ferns, Dicksoniaceae was recovered as monophyletic. However, including *Cystodium* in Dicksoniaceae, has been challenged based on differences in its stipe anatomy (Nishida, 1984; Croft, 1986) and spore morphology (Gastony, 1981). Croft (1986) even considered *Cystodium* to be so distinctive from other dicksonioid genera that it merited its own family, Cystodiaceae. Here we use DNA sequence data from two plastid genes (*rbcL* and *atpB*) to investigate the phylogenetic relationship of *Cystodium* to other leptosporangiate ferns, and specifically to address whether it belongs within tree ferns.

Materials and Methods

Taxon sampling, DNA isolation, amplification, sequencing, and sequence alignment.—Total DNA of *Cystodium sorbifolium* was extracted using the DNeasy plant mini kit from Qiagen (Valencia, California, USA). The voucher specimen is *Christensen 1529* (S) (record no. 2498 in Fern DNA database at http://www.biology.duke.edu/pryerlab/ferndb). The *rbcL* and *atpB* genes from the plastid genome were amplified using the polymerase chain reaction (PCR), following standard protocols. PCR products were cleaned using the Montage PCR cleanup kit (Millipore, Billerica, Massachusetts, USA) according to the manufacturer's protocol. Sequencing reactions were carried out for both strands of the purified PCR products using Big Dye Terminator Cycle Sequencing reagents (Applied Biosystems, Foster City, California, USA). For information on amplification and sequencing primers, see Table 1. All sequencing reactions were processed using either ABI 3700 or ABI 3730XL automated sequencers (Applied Biosystems). Sequence fragments were assembled and edited using Sequencher version 4.2.2 (Gene Codes, Ann Arbor, Michigan, USA). The corrected consensus sequences were aligned manually using MacClade version 4.07b13 (Maddison and Maddison, 2005). The *rbcL* and *atpB* Genbank numbers for *C. sorbifolium* are AM184111 and

TABLE 1. Primers used for amplifying and sequencing *rbcL* and *atpB* from *Cystodium sorbifolium*.

Primer	Sequence (5′ to 3′)	References
rbcL		
ESRBCL1F	ATGTCACCACAAACGGAGACTAAAGC	Korall *et al.*, 2006
ES645F	AGAYCGTTTCYTATTYGTAGCAGAAGC	Korall *et al.*, 2006
ES663R	TACRAATARGAAACGRTCTCTCCAACG	Korall *et al.*, 2006
ESRBCL1361R	TCAGGACTCCACTTACTAGCTTCACG	Korall *et al.*, 2006
atpB		
ATPB672F	TTGATACGGGAGCYCCTCTWAGTGT	Wolf, 1997
ATPB1163F	ATGGCAGAATRTTTCCGAGATRTYA	Wolf, 1997
ATPB1419F	CRACATTTGCACATYTRGATGCTAC	Wolf, 1997
ATPB1592R	TGTAACGYTGYAAAGTTTGCTTAA	Wolf, 1997
ATPB609R	TCRTTDCCTTCRCGTGTACGTTC	Pryer *et al.*, 2004
ATPE384R	GAATTCCAAACTATTCGATTAGG	Pryer *et al.*, 2004

AM184112, respectively. All *rbcL* and *atpB* sequences for the other 62 taxa included here were taken from Pryer *et al.* (2004). No insertions or deletions (indels) were required to align *rbcL* or *atpB*.

Phylogenetic analyses.—The *rbcL* and *atpB* data sets were analysed with a Bayesian Markov Chain Monte Carlo approach (B/MCMC) using the parallel version of MrBayes 3.0B4 (Ronquist and Huelsenbeck, 2003), and equally weighted maximum parsimony (MP) with PAUP* version 4.0 beta 10 (Swofford, 2002). All analyses were performed on the CSEM/OIT high-performance, shared computing cluster at Duke University (Durham, North Carolina, USA). Trees were rooted with three lycopods: *Huperzia* Bernh., *Selaginella* Pal. Beauv., and *Isoetes* L. (Pryer *et al.*, 2004).

Nucleotide substitution models for the Bayesian analyses were chosen using MrModeltest 2.2 (a modified version of Modeltest 3.6; Posada and Crandall, 1998). Each gene was treated as a single partition (following Pryer *et al.*, 2004), and for both *rbcL* and *atpB* the general time reversible model (GTR) with a gamma distribution of substitution rates (Γ) and a proportion of invariant sites (I) was suggested based on the Akaike information criterion (AIC). Separate analyses of the two datasets were run for 3 million generations, on six parallel chains, with the temperature parameter (for heating the chains) set to 0.1. The sampled values for different parameters were examined using MrBayes and Tracer v. 1.2.1 (Rambaut and Drummond, 2005) to see if the parameters had converged. We also checked for a proper mixing of the chains. For each analysis every 1000th tree was sampled and, after analysing parameter values, 300 trees were discarded as "burn-in". Each analysis was repeated four times to ascertain that apparent stationarity was reached. The majority rule consensus trees from the replicates were compared and no differences were found. Trees from each analysis were then pooled (a total of 10800 trees, excluding those discarded as burn-in) before a majority rule consensus tree was calculated. In our Bayesian analyses, we consider branches with a posterior probability (PP) of 1.00 as well supported, a PP between 0.95–0.99 as moderately supported, and a PP of <0.95 as low support.

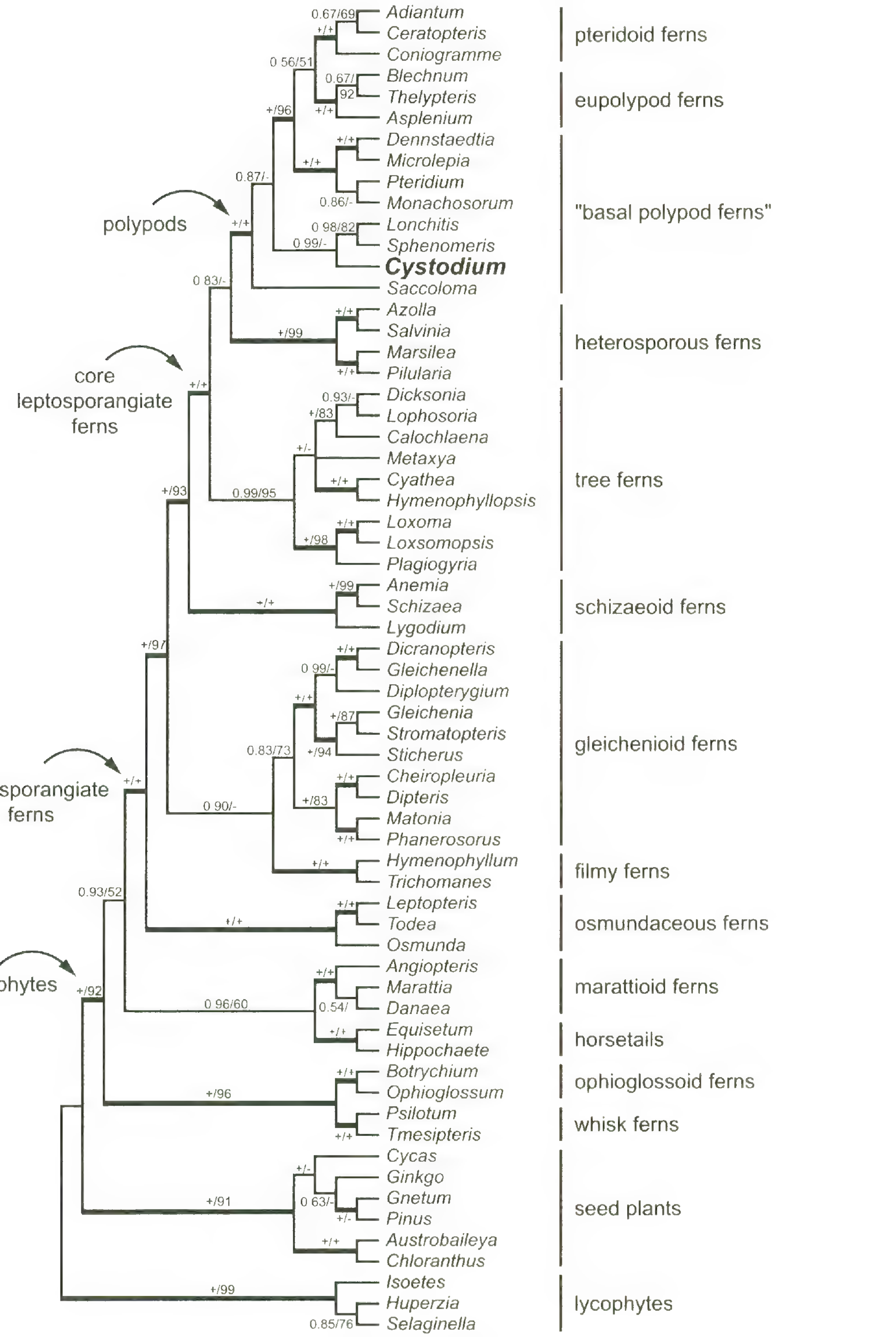


FIG. 1. 50% majority-rule consensus tree resulting from Bayesian (B/MCMC) analyses of the combined (*rbcL* and *atpB*) data set. Numbers on branches denote support values from Bayesian

MP analyses for each data set included a heuristic search for the most parsimonious trees with 1000 random-sequence-addition replicates, and TBR branch swapping. Support for nodes was calculated by a bootstrap analysis, with 3000 replicates, each with 10 random-sequence-addition replicates, and TBR branch swapping. In our MP analyses, we considered branches with a bootstrap percentage (BP) of $\geq$90% as well-supported, 70–89% as moderately supported, and <70% as weakly supported.

Combinability of rbcL *and* atpB *data sets.*—To evaluate combinability of data sets, the resultant topologies from each of the single-gene analyses were compared and examined for potential conflicts. Comparisons were made between topologies produced by the same analytical method, i.e., B/MCMC was compared with B/MCMC, and MP with MP. Incongruence supported by a MP bootstrap percentage of 70 or higher, or a Bayesian posterior probability of 0.99 or higher, was considered a conflict. Because no conflicts were observed, the two data sets were combined into a single data set.

Analyses of the combined data set.—The combined data set was analysed with settings as for the separate data sets. In the B/MCMC analyses, the two genes were each treated as one partition (i.e., a total of two partitions) and each was assigned the GTR + Γ + I model of nucleotide substitution (as in the separate data set analyses).

Results

Tree statistics.—All three data sets (*rbcL, atpB*, and combined) included 63 taxa. The *rbcL* data set included 1320 characters of which 559 were parsimony informative, the *atpB* data set 1150 characters (562 parsimony informative), and the combined data set 2470 characters (1121 parsimony informative). In MP, the *rbcL* analysis yielded two most parsimonious trees, with a tree length of 4788 steps, found in one island; the *atpB* analysis yielded 47 trees of 4088 steps in one island; the combined analysis resulted in eight trees of 8895 steps found in two islands (trees not shown).

Conflicting topologies.—Conflicts in leptosporangiate fern relationships (i.e., incongruence supported by a BP $\geq$ 70% or PP $\geq$ 0.99) were not found when MP topologies from the separate analyses of *atpB* and *rbcL* were compared to each other, or when B/MCMC analyses were compared to each other. The only conflict found was when comparing between analytical methods *within* the combined analysis: in the tree fern clade, *Metaxya* is sister to the other tree ferns in MP (BP = 89%), but is nested within the clade with strong support in B/MCMC (PP = 1.00).

←

(posterior probability; PP) and maximum parsimony (bootstrap percentages; BP) analyses. A plus (+) represents a PP = 1.00, or BP = 100%. A dash (–) represents a bootstrap percentage <50%. Thickened branches are well supported (PP = 1.00, BP $\geq$ 90%). Names of clades follow Pryer *et al.* (2004) and Schneider *et al.* (2004).

Phylogenetic relationships of leptosporangiate ferns.—Because the resultant topologies for leptosporangiate relationships from each of the separate gene analyses were not in conflict with one another, the phylogenetic relationships presented here are based on analyses of the combined data set (Fig. 1).

Cystodium, the focus of our study, is resolved as part of the polypod fern clade with high support (PP = 1.00, BP = 100%; Fig. 1). Within polypods, there is a weakly supported basal split (PP = 0.87, BP < 50%) with *Saccoloma* Kaulf. sister to the rest. The subsequent divergence yields a clade where *Cystodium* is sister to *Lonchitis* L. + *Sphenomeris* Maxon (PP = 0.99, BP < 50%), and together these taxa are sister to a large clade that includes the well-supported pteridoid, eupolypod, and a more exclusive group of basal polypod ferns (PP = 1.00, BP − 96%).

The polypods are included in the core leptosporangiate ferns (PP = 1.00, BP = 100%) together with the tree ferns (PP = 0.99, BP = 95%) and the heterosporous ferns (PP = 1.00, BP − 99%). The core leptosporangiate ferns are part of the well-supported leptosporangiate ferns (PP = 1.00, BP = 100%) as sister to the strongly supported schizaeoid ferns (PP = 1.00, BP = 100%). Filmy ferns and gleichenioid ferns group together with low support (PP = 0.90, BP < 50%) and the osmundaceous ferns are strongly supported as sister to the rest of leptosporangiate ferns (PP = 1.00, BP = 97%; Fig. 1).

Discussion

Conflicting topologies.—In the combined analysis, the conflicting positions of *Metaxya* within the tree ferns between analytical methods (MP vs. B/MCMC) was also recovered in a study on tree ferns (Korall *et al.*, 2006) and is discussed there as likely being due to a long branch attraction artefact (Felsenstein, 1978) in the MP analysis.

Phylogenetic relationships of leptosporangiate ferns, Cystodium *in particular.*—The overall leptosporangiate fern relationships shown in Fig. 1 are not in conflict with the results of Pryer *et al.* (2004). Our results clearly demonstrate that *Cystodium* is not a tree fern—it is a polypod (Fig. 1). *Cystodium* is likely closely related to *Sphenomeris* and *Lonchitis* (or perhaps *Saccoloma*), and is not included in the well-supported and species-rich clade that includes the pteridoid, eupolypod, and a more exclusive group of basal polypod ferns (clade names sensu Schneider *et al.*, 2004).

Several morphological and anatomical studies have revealed distinctive differences between *Cystodium* and other dicksonioid genera (Sen and Mittra, 1966; Gastony, 1981; Nishida, 1984; Croft, 1986; Tryon and Lugardon, 1991), leading some authors to suggest a close affinity between *Cystodium* and *Saccoloma* and/or *Dennstaedtia* Bernh., for example.

Sen and Mittra (1966) listed several features to separate *Cystodium* from *Dicksonia* (including receptacle shape, cortex structure, and orientation of subsidiary and guard-mother cells), but they nevertheless considered it to be part of the dicksonioid-cyathaeoid group.

Their conclusion was contradicted by Nishida (1984) and Croft (1986) who thoroughly studied the stipe vasculature of *Cystodium* and showed major differences with other dicksonioid genera. In cross section the stipe vasculature of *Cystodium* consists of several strands that unite to first form two bilateral branched arcs, and then, closer to the lamina, two dorsiventral, branched arcs. In *Dicksonia* several vascular bundles form one abaxial and two lateral arcs, which unite into a single incurved (U-shaped) strand. Other dicksonioid genera have vascular strands similar to *Dicksonia*. This led Croft (1986) to transfer *Cystodium* to a separate, monotypic family, Cystodiaceae, and to suggest that *Cystodium* be compared to "other groups with 2-lipped indusia and hairy rhizomes, such as the Dennstaedtiaceae" (in the broad sense, e.g., following Kramer, 1990).

Croft (1986) also noted that the annulus of *Cystodium* sporangia is not fully oblique and continuous, as is typical of other dicksonioid genera. It should be noted, however, that there is no general agreement on how to interpret the tree fern annulus; one morphological cladistic study considers the annulus to be oblique (Stevenson and Loconte, 1996), whereas another treats it as vertical to slightly oblique (i.e., as in the polypods; Pryer *et al.*, 1995).

Further supporting a relationship with the Dennstaedtiaceae *s.l.* is the striated perine of the spores. *Cystodium* shares this spore ornamentation with *Saccoloma* and some species of *Dennstaedtia* (Gastony, 1981; Tryon and Lugardon, 1991).

In summary, several morphological and anatomical studies have suggested significant differences between *Cystodium* and other genera traditionally assigned to Dicksoniaceae. In addition, these studies propose some similiarities to Dennstaedtiaceae *s.l.* In order to gain a better understanding of the precise phylogenetic position of *Cystodium*, the taxon sampling of early diverging lineages of polypods will need to be greatly expanded. Until then, we advocate the tentative inclusion of *Cystodium* in Lindsaeaceae, following a new classification for extant ferns (Smith *et al.*, 2006), as it is certainly not a member of Dicksoniaceae.

Acknowledgments

This study was financially supported by a postdoctoral fellowship to Petra Korall from the Swedish Research Council (2003-2724) and a NSF CAREER grant to Kathleen Pryer (DEB-0347840). Herbarium curators at S are thanked for their help.

Literature Cited

Christensen, C. 1938. Filicinae. *In* F. Verdoorn, ed., *Manual of Pteridology*, 522–550. Martinus Nijhoff, The Hague.

Copeland, E. B. 1947. *Genera Filicum*. Chronica Botanica Company, Waltham, MA, USA.

Croft, J. R. 1986. The stipe and rachis vasculature of the dicksonioid fern, *Cystodium sorbifolium* (Cystodiaceae). Kew Bull. 41:789–803.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27:401–410.

GASTONY, G. J. 1981. Spore morhology in the Dicksoniaceae. I. The genera *Cystodium, Thyrsopteris,* and *Culcita.* Amer. J. Bot. 68:808–819.

HASEBE, M., T. OMORI, M. NAKAZAWA, T. SANO and M. KATO. 1994. *rbcL* gene sequences provide evidence for the evolutionary lineages of leptosporangiate ferns. Proc. Natl. Acad. Sci. USA 91:5730–5734.

HASEBE, M., P. G. WOLF, K. M. PRYER, K. UEDA, M. ITO, R. SANO, G. J. GASTONY, J. YOKOYAMA, J. R. MANHART, N. MURAKAMI, E. H. CRANE, C. H. HAUFLER and W. D. HAUK. 1995. Fern phylogeny based on *rbcL* nucleotide sequences. Amer. Fern J. 85:134–181.

HOLTTUM, R. E. and U. SEN. 1961. Morphology and classification of the tree ferns. Phytomorphology 11:406–420.

HOLTTUM, R. E. 1963. Cyatheaceae. *In* C. G. G. J. Van Steenis and R. E. Holttum, eds., *Flora Malesiana.* Martinus Nijhoff, Dr. W. Junk Publishers, The Hague, Boston, London.

HOOKER, W. J. 1844. *Species Filicum. Vol. II.* William Pamplin, London.

KORALL, P., K. M. PRYER, J. S. METZGAR, H. SCHNEIDER and D. S. CONANT. 2006. Tree ferns: monophyletic groups and their relationships as revealed by four protein-coding plastid loci. Molec. Phylogenet. Evol. 39:830–845

KRAMER, K. U. 1990. Dicksoniaceae. *In* K. Kubitzki, ed., [Vol. eds. K. U. Kramer and P. S. Green]. *The families and genera of vascular plants. Vol. 1 Pteridophytes and Gymnosperms.* Springer-Verlag, Berlin, Germany.

MADDISON, D. R. and W. P. MADDISON. 2005. *MacClade version 4.07.* Sinauer Associates, Sunderland, Massachusetts, USA.

NISHIDA, H. 1984. Anatomical studies of the frond axis of the Cyatheaceae, *s.l.*, with a revision of permineralized frond axes from the Cretaceous of Japan. *In* M. Nishida, ed., *Contributions to the Botany in the Andes I,* 5-80. Academia Scientific Book, Tokyo.

PICHI SERMOLLI, R. E. G. 1977. Tentamen Pteridophytorum. Webbia 31:315–512.

POSADA, D. and K. A. CRANDALL. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:817–818.

PRYER, K. M., A. R. SMITH and J. E. SKOG. 1995. Phylogenetic relationships of extant ferns based on evidence from morphology and *rbcL* sequences. Amer. Fern J. 85:205–282.

PRYER, K. M., H. SCHNEIDER, A. R. SMITH, R. CRANFILL, P. G. WOLF, J. S. HUNT and S. D. SIPES. 2001. Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. Nature 409:618–622.

PRYER, K. M., E. SCHUETTPELZ, P. G. WOLF, H. SCHNEIDER, A. R. SMITH and R. CRANFILL. 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on the early leptosporangiate divergences. Amer. J. Bot. 91:1582–1598.

RAMBAUT, A. and A. DRUMMOND. *Tracer version 1.2.1.* Computer program distributed by the authors. Department of Zoology, University of Oxford. UK.

RONQUIST, F. and J. P. HUELSENBECK. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574.

ROTHWELL, G. W. 1999. Fossils and ferns in the resolution of land plant phylogeny. Bot. Rev. 65:188–218.

SCHNEIDER, H., E. SCHUETTPELZ, K. M. PRYER, R. CRANFILL, S. MAGALLÓN and R. LUPIA. 2004. Ferns diversified in the shadow of angiosperms. Nature 428:553–557.

SEN, U. and D. MITTRA. 1966. The anatomy of *Cystodium.* Amer. Fern J. 56:97–101.

SMITH, A. R., K. M. PRYER, E. SCHUETTPELZ, P. KORALL, H. SCHNEIDER and P. G. WOLF. 2006. A classification for extant ferns. Taxon 55, in press.

STEVENSON, D. W. and H. LOCONTE. 1996. Ordinal and familial relationships of pteridophyte genera. Pp. 435–467 *in* J. M. Camus, M. Gibby and R. J. Johns, eds., *Pteridology in perspective.* Royal Botanic Gardens, Kew.

SWOFFORD, D. L. 2002. *PAUP*: phylogenetic analysis using parsimony (*and other methods).* Sinauer Associates, Sunderland, Massachusetts, USA.

TRYON, A. F. and B. LUGARDON. 1991. *Spores of the Pteridophyta: surface, wall structure, and diversity based on electron microscope studies.* Springer-Verlag, New York.

TRYON, R. M. and A. F. TRYON. 1982. *Ferns and allied plants, with special reference to tropical America.* Springer-Verlag, New York.

WIKSTRÖM, N. and K. M. PRYER. 2005. Incongruence between primary sequence data and the distribution of a mitochondrial *atp1* group II intron among ferns and horsetails. Mol. Phylogenet. Evol. 36:484–493.

WOLF, P. G. 1997. Evaluation of *atpB* nucleotide sequences for phylogenetic studies of ferns and other pteridophytes. Amer. J. Bot. 84:1429–1440.

WOLF, P. G., S. D. SIPES, M. R. WHITE, M. L. MARTINES, K. M. PRYER, A. R. SMITH and K. UEDA. 1999. Phylogenetic relationships of the enigmatic fern families Hymenophyllopsidaceae and Lophosoriaceae: evidence from *rbcL* nucleotide sequences. Pl. Syst. Evol. 219:263–270.

American Fern Journal 96(2):54–61 (2006)

Gametophytes of Four Tropical, Terrestrial *Huperzia* Species (Lycopodiaceae)

Dean P. Whittier
Department of Biological Sciences, Box 1634,
Vanderbilt University, Nashville, TN 37235-1634

Abstract.—The gametophytes of four tropical, terrestrial species of *Huperzia* – *H. crassa*, *H. cumingii*, *H. hypogaea*, and *H. saururus* – grow in axenic culture on a nutrient medium containing inorganic nutrients and glucose. The gametophytes of all species are dorsiventral, axial structures, which can be straight, curved, narrow, or wide. Paraphyses and gametangia form on the dorsal surface and rhizoids on the ventral surface. The apical meristem is overarched by immature dorsal tissue. Minor differences in the paraphyses and gametangia exist among the species. These gametophytes are Type III gametophytes as is the case for the previously described gametophytes of two other terrestrial species of *Huperzia*.

Gametophytes of the Lycopodiaceae are known from less than 10% of the species (Bruce and Beitel, 1979). The subterranean, mycorrhizal gametophytes of this group are especially difficult to find. With axenic culture the subterranean gametophytes of this family can be grown on nutrient media containing minerals and sugar (Whittier, 1977, 1981; Whittier and Webster, 1986).

Gametophytes of three species representing three of the four types of subterranean, mycorrhizal gametophytes described by Bruchmann (1898, 1910) for the Lycopodiaceae have been grown in culture (Whittier, 1977, 1981; Whittier and Webster, 1986). Under these conditions, gametophytes of these three species have essentially the same structure as those collected from nature. The absence of the mycorrhizal fungus from these cultured gametophytes had no significant effect on their development.

Because gametophytes from so few species of the Lycopodiaceae are known, information on more of them is needed to provide a better understanding of the gametophytes in this family. Axenic culture provides an opportunity to make undescribed gametophytes available for study. In this study gametophytes of four *Huperzia* species are described for the first time.

Materials and Methods

Gametophytes from a study on spore germination in the Lycopodiaceae provided the material for this study (Whittier, 1998). Spores from four *Huperzia* species provided numerous mature gametophytes in culture. The gametophytes grown were those of *H. crassa* (Willd.) Rothm. var. *gelida* B. Øllg., *H. cumingii* (Nessel) Holub, *H. hypogaea* B. Øllg., and *H. saururus* (Lam.) Trevisan. Spores from the first three species were obtained in Ecuador and those of *H. saururus* came from Kenya. Vouchers of the sporophytes are on

deposit at AAU and AK. The system of classification followed in this study is that of Øllgaard (1987, 1989).

The spores germinated (Whittier, 1998) and the young gametophytes grew on 14ml of nutrient medium in culture tubes (20 × 125 mm) with screw caps that were tightened to reduce moisture loss. The cultures were maintained at 22 ± 1°C. After nine months the young gametophytes were transferred to fresh nutrient medium and they were grown to maturity. Except for the brief time when the young gametophytes were being transferred, the gametophytes were maintained in the dark. Mature gametophytes were obtained 2–3 years after sowing the spores.

The nutrient medium contained 50 mg NH_4Cl_2, 50 mg $MgSO_4 \cdot 7H_2O$, 25 mg $CaCl_2$, and 50 mg K_2HPO_4 per liter. Trace elements and FeEDTA completed the mineral composition of this medium (Whittier, 1998). Glucose (0.2%) was the carbon source for these nonphotosynthetic gametophytes. The medium was solidified with 1% agar and its pH was 5.1 ± 0.1 after autoclaving.

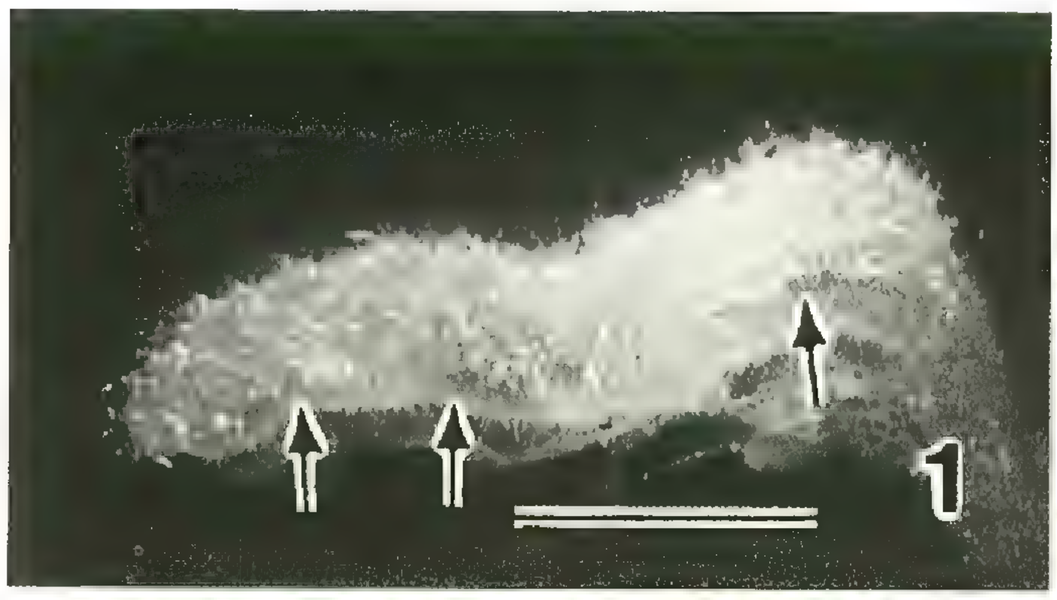
For light microscopy the gametophytes were fixed in Randolph's modified Navashin fluid (CRAF). After fixation, the gametophytes were embedded in paraffin and sectioned by conventional techniques (Johansen, 1940). The sections were stained with Heidenhain's hematoxylin, safranin O, and fast green. For scanning electron microscopy, the gametophytes were fixed overnight on ice in a 1:1 solution of 4% glutaraldehyde and 10% acrolein in 0.1 M Hepes buffer (pH 6.8). The gametophytes were postfixed with 1% osmium tetroxide in 0.1 M Hepes buffer (pH 6.8) at room temperature for one hour. They were then treated with 1% aqueous thiocarbohydrazide for 30 minutes after the osmium postfixation. The gametophytes were refixed with 2% osmium tetroxide in water for 1 hour and dehydrated in a graded acetone series. All specimens were critical point dried and coated with gold-palladium before observing with a Hitachi 4500 scanning electron microscope at 10 KV.

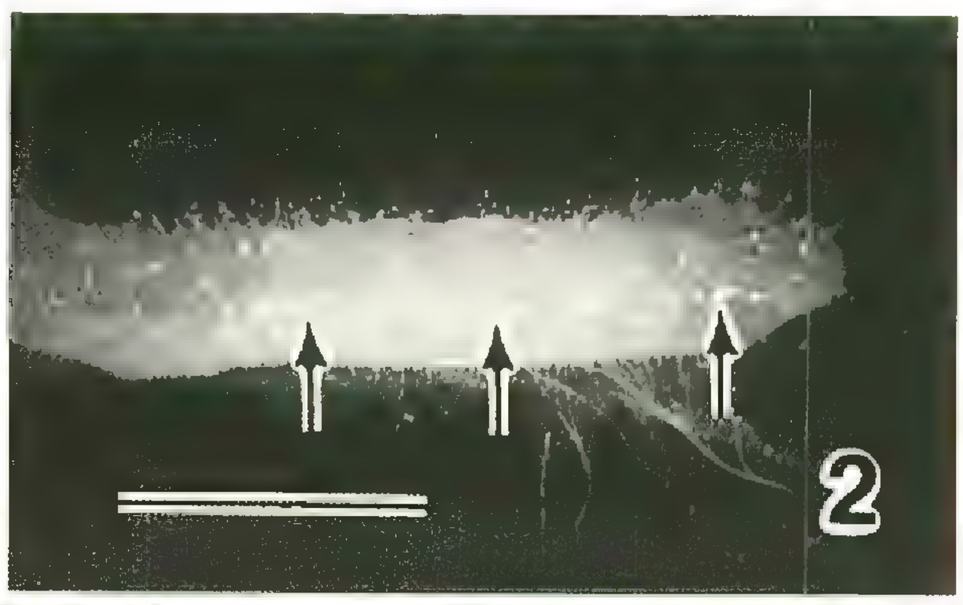
Results

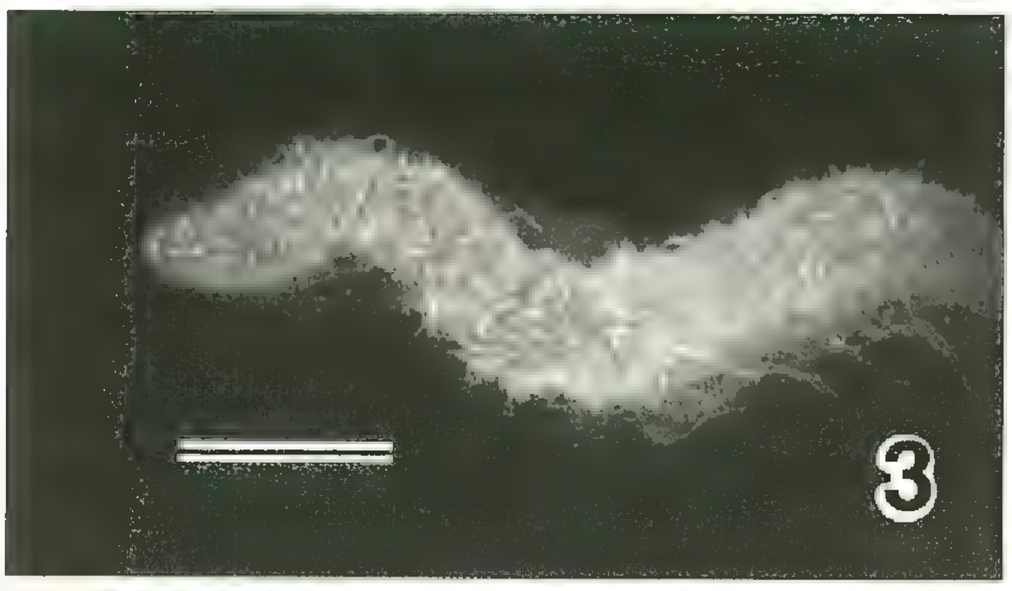
The mature gametophytes of these species are axial structures but not cylindrical (Figs. 1–7). They are long and can be narrow or wide and very few branch. The wider gametophytes have a thickened strap shape. The terminal growth can change directions so that not all the gametophytes are straight (Figs. 1, 3–6). Some gametophytes have sharp bends and others are sinuate with repeated minor bends. No distinctive differences in the gross morphology are noticeable among these gametophytes as grown in culture.

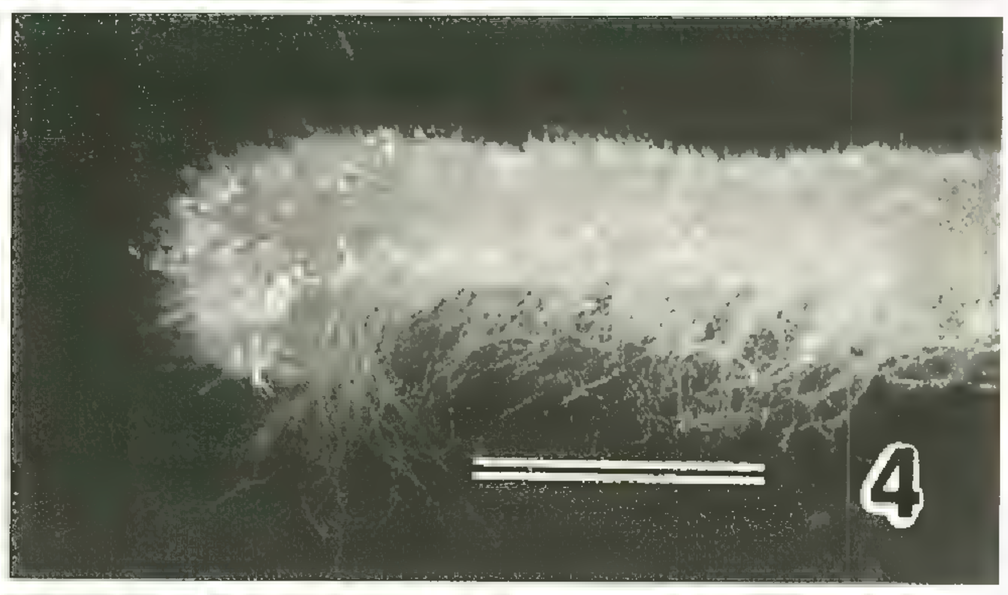
Even long narrow gametophytes that appear to be cylindrical to the eye are not (Fig. 2). All gametophytes, narrow or wide, have dorsal and ventral surfaces that are separated by indentations along the sides of the gametophytes (Figs. 1–2). These indentations give the narrower gametophytes a more or less hourglass shape in cross section (Fig. 8).

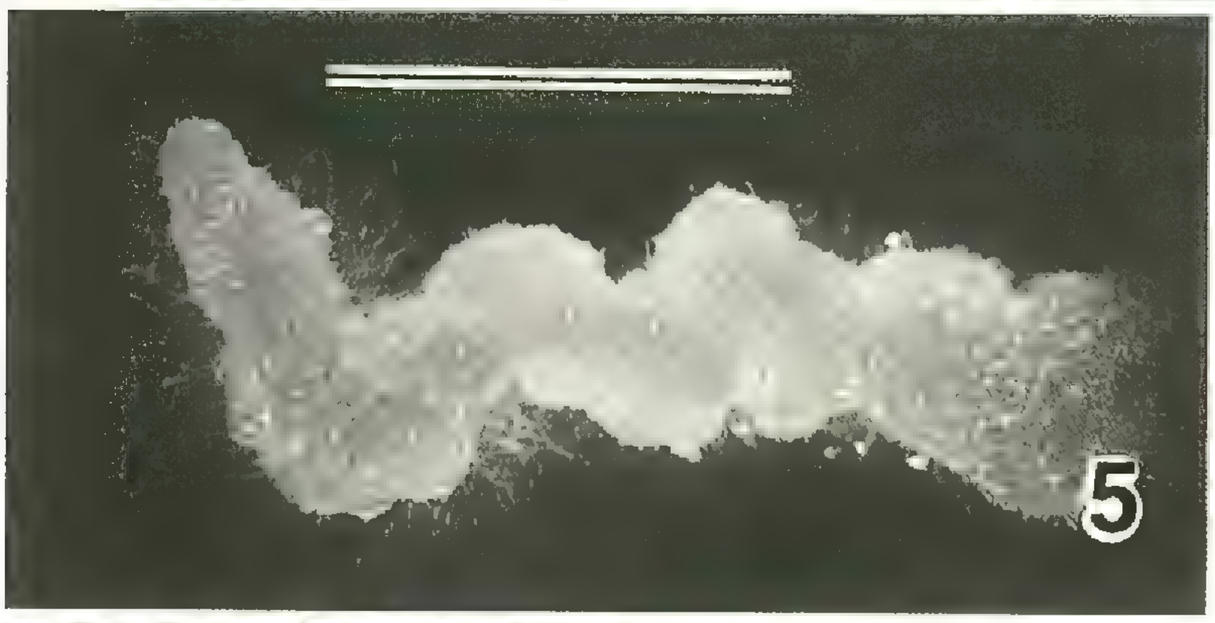
Large numbers of absorbing rhizoids occupy the ventral surface of the gametophytes (Figs. 1, 2, 4–6). These rhizoids have mucilaginous sheaths that contain acid mucopolysaccharides (not illustrated). The gametangia form on

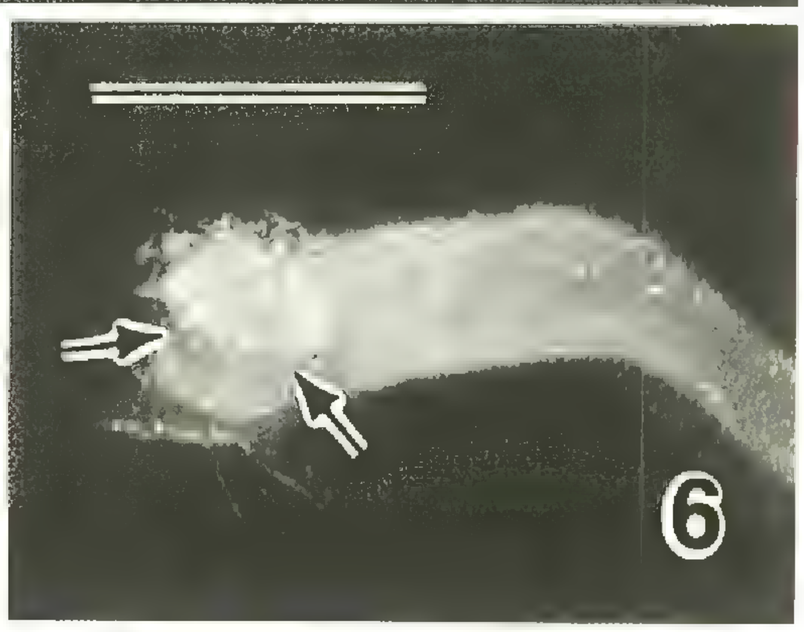

1


2

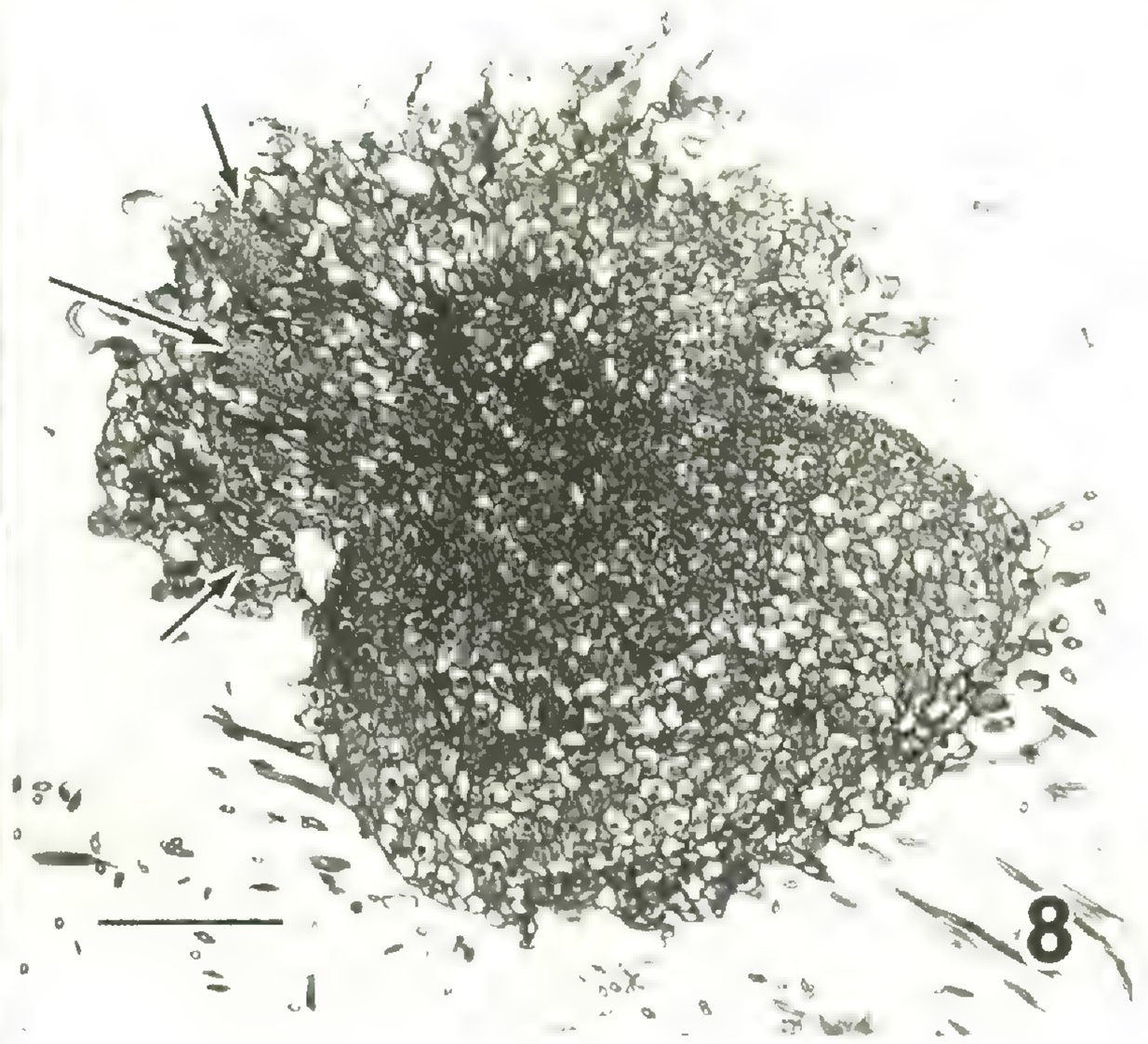

3


4


5


6

7


8

the dorsal surface (Figs. 8–11) along with the paraphyses which make viewing the antheridia and archegonia difficult.

The apical meristem is in a groove on the lower surface of the apical end of the gametophyte (Figs. 2, 6). The derivatives to the upper side form the dorsal tissues including the gametangia and paraphyses. The immature dorsal tissues overarch the meristematic region and cause the meristematic groove to be on the lower surface of the apex (Figs. 2, 6, 9, 11). The meristematic derivatives to the lower side form the ventral portion of the gametophyte including the rhizoids. The meristematic groove lines up with the lateral indentations on the sides of the gametophyte.

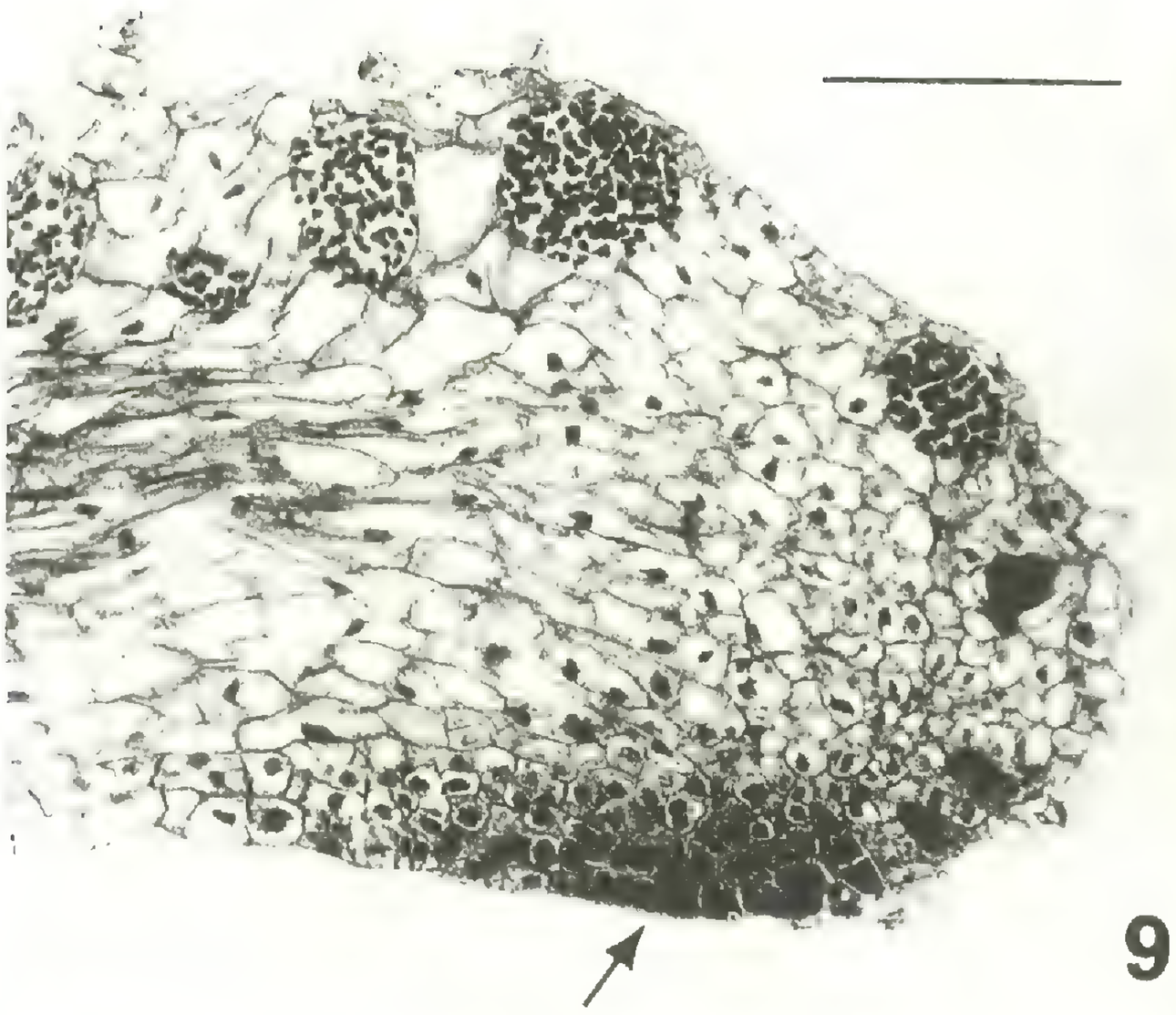
The apical region is usually the same width as the mature portion of the gametophyte, especially the region immediately basipetal to the apex (Figs. 3, 6). It appears that there is little meristematic activity on the lateral margins of the meristematic groove in the apical region.

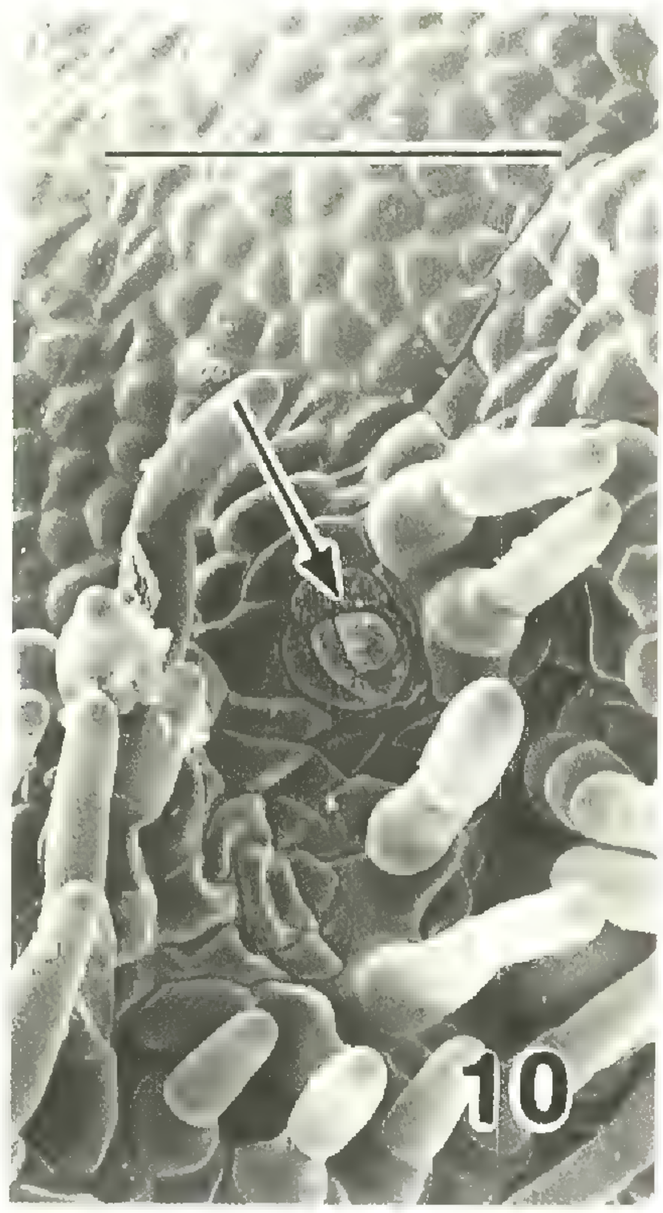
The gametophytes of these species have the same basic structure in culture. However, some differences are noted with the sizes of the paraphyses, antheridia, and archegonia among the species. The paraphyses are normally unicellular or uniseriate filaments (Figs. 7, 10) although occasionally there may be a biseriate base supporting two uniseriate filaments. The morphologies of the filaments are essentially the same among the species, however there are size differences (Table 1). The length of the paraphyses varies and those of *H. hypogaea* are more than twice as long as the others. There are fewer cells per paraphysis in *H. crassa* and *H. saururus* because they have many unicellular paraphyses (Table 1). The cell lengths in the paraphyses are the smallest in *H. crassa*.

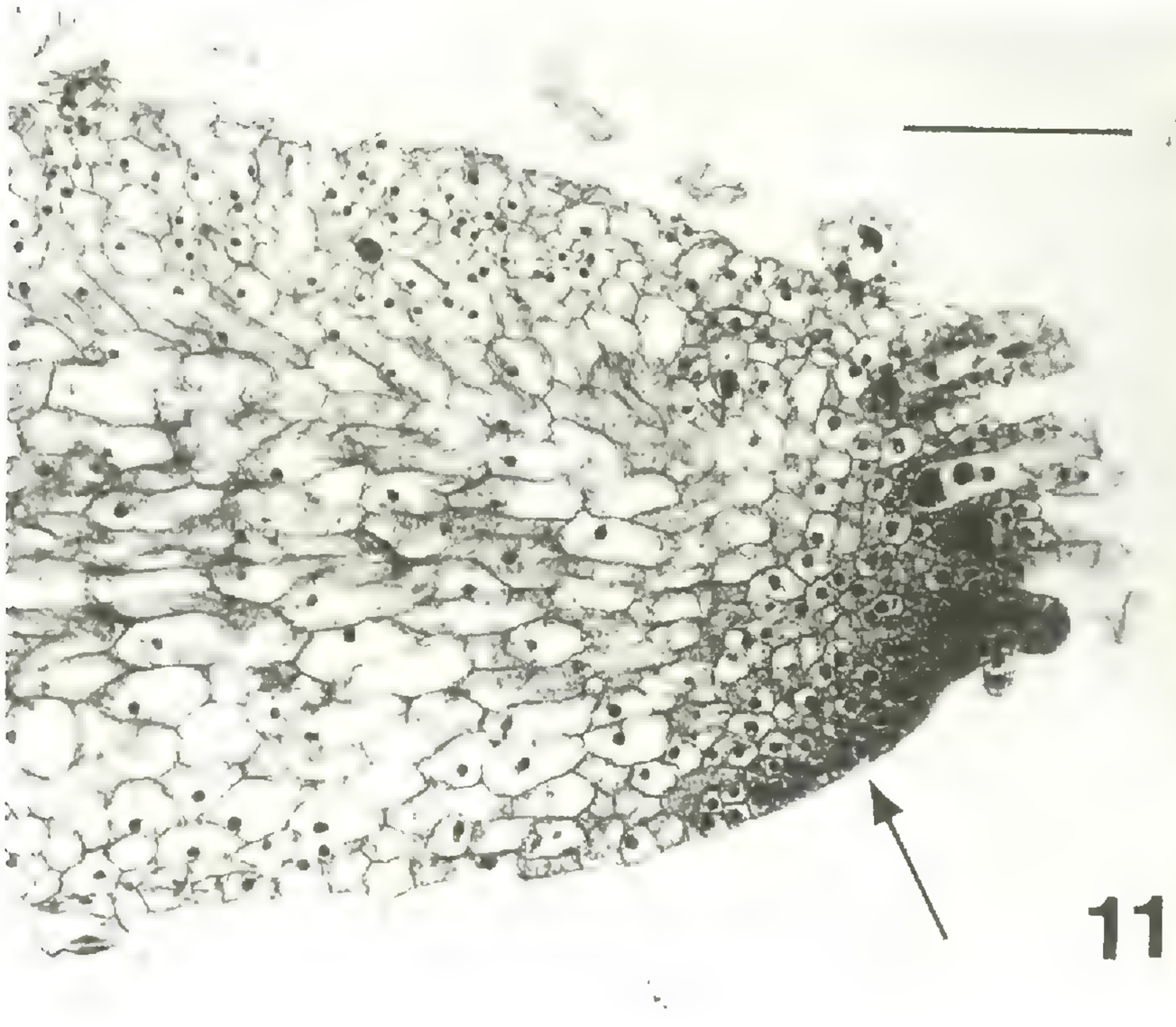
The antheridia (Fig. 9) have the same structure for all the species. They contain ellipsoidal masses of gametes and one opercular cell in the jacket layer at the gametophyte surface. The opercular cell of *H. cumingii* has a triangular face (Fig. 10). Some variation occurs in the sizes of the gamete mass (Table 2). The average lengths range from 130.7 μm to 92.2 μm with *H. cumingii* having the longest and *H. crassa* having the shortest. The average diameter at the widest point ranges from 77.8 μm for *H. cumingii* to 54.0 μm for *H. hypogaea*. The antheridia of *H. cumingii* are the largest because they are longest and widest.

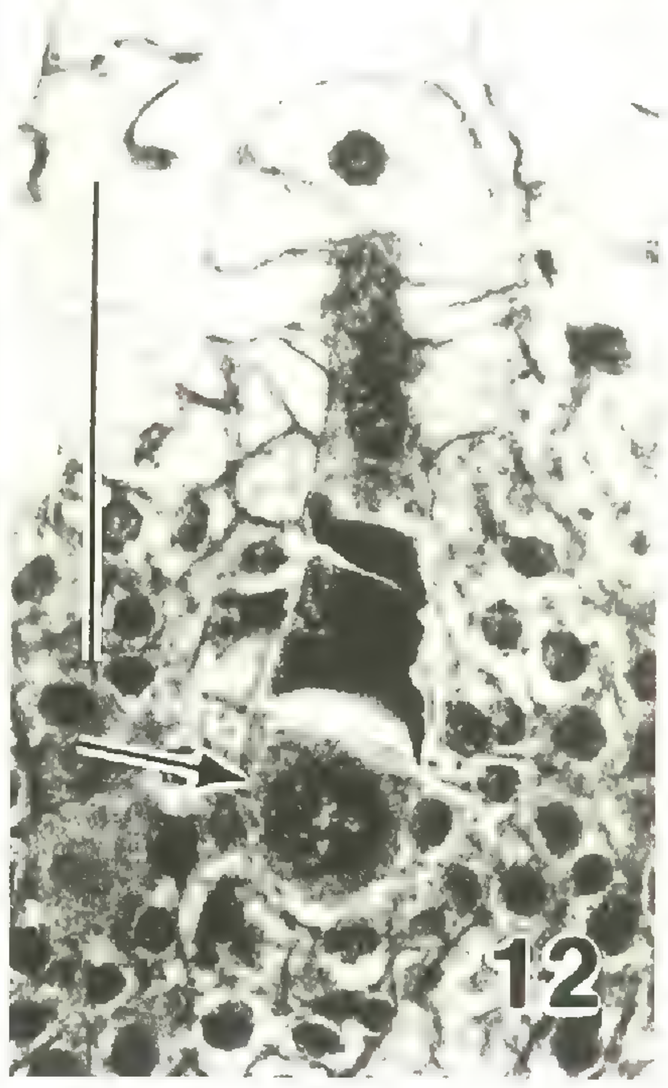
←

FIGS. 1–8. Tropical terrestrial *Huperzia* gameophytes. 1. Lateral view of sinuous gametophyte of *H. hypogaea* with a paraphysis-bearing dorsal surface and a lateral indentation (arrows). 2. Lateral view of straight gametophyte of *H. hypogaea* with paraphysis-bearing dorsal surface, rhizoid-bearing ventral surface, lateral indentation (arrows) and overarching dorsal tissue in apical region. 3. Dorsal view of sinuous gametophyte of *H. hypogaea*. 4. Lateral view of gametophyte of *H. cumingii* with downward growing apical region. 5. Dorsal view of sinuous gametophyte of *H. crassa*. 6. Apical view of gametophyte of *H. saururus* with meristematic groove (arrows) below overarching dorsal tissue. 7. Paraphyses on dorsal surface of gametophyte of *H. cumingii*. 8. Cross section of gametophyte of *H. cumingii* with lateral indentations separating antheridia-bearing dorsal region (arrows) from rhizoid-bearing ventral region. Bars = 3 mm for Figs. 1–6 and 250 μm for Figs. 7–8.









FIGS. 9–12. Structural details of tropical terrestrial *Huperzia* gametophytes. 9. Sagittal section through apical region of gametophyte of *H. crassa* with meristem (arrow) and developing antheridia in dorsal tissues. 10. Surface view of gametophyte of *H. cumingii* at edge of dorsal region with paraphyses and the opercular cell of an antheridium (arrow). 11. Sagittal section through apical region of gametophyte of *H. hypogaea* with meristem (arrow) and developing archegonia. 12. Longitudinal section of archegonium of *H. cumingii* with egg (arrow) and neck canal cells. Bars = 200 μm for Figs. 9–11 and 100 μm for Fig. 12.

TABLE 1. Paraphysis structure in *Huperzia*.

Species	Length (μm)	Cells per paraphysis	Average number of cells per paraphysis
H. crassa	82.8	1–2	1.5
H. cumingii	166.5	2–4	2.8
H. hypogaea	264.2	3–8	3.9
H. saururus	115.6	1–2	1.6

The archegonia are normal for the Lycopodiaceae (Fig. 12) and their measurements appear in Table 2. The length of the archegonium is measured from the base of the egg to the tip of the neck. The archegonial lengths are similar for these species with those of *H. cumingii* being the longest at an average of 234.1 μm. However, the long archegonia of *H. cumingii* do not have necks that protrude the most above the gametophyte surface. The necks of *H. hypogaea* have the greatest protrusion with an average of 109.2 μm. For these species the tiers of neck cells are 4 or 5 and the number of cells in the neck canal above the eggs is 3 or 4.

DISCUSSION AND CONCLUSIONS

The gametophytes of these four species are essentially the same. They are axial, strap-shaped gametophytes with dorsal and ventral surfaces. Paraphyses and gametangia form on the dorsal surface and rhizoids on the ventral surface. The meristematic groove at the apical end of the gametophyte is overarched by the developing dorsal tissues. Gametophytes from all species could be straight, curved, narrow or wide. None are cylindrical. As would be expected the gametophytes from culture lack mycorrhizal fungi. The gametophytes of *H. crassa*, *H. cumingii*, *H. hypogaea* and *H. saururus* are basically the same as those of *Lycopodium lucidulum* (=*H. lucidula*) from culture (Whittier and Webster, 1998).

Under these growing conditions, the only consistent variations among these gametophytes are those associated with the paraphyses and gametangia. The length and number of cells in the unicellular or uniseriate paraphyses are different from species to species. The paraphyses of *H. hypogaea* are the

TABLE 2. Gametangial structure in *Huperzia* with sizes in μm.

	Antheridia	Archegonia			
Species	Gamete Mass width/length	Length: egg base to neck tip	Neck protrusion	Average number of neck cells	Number of neck cells
H. crassa	60.7/92.2	212.2	88.7	4.0	—
H. cumingii	77.8/130.7	234.1	95.3	4.7	4
H. hypogaea	54.0/100.9	207.5	109.2	5.0	4
H. saururus	70.4/104.2	189.5	90.5	4.0	3

longest with the largest number of cells and those of *H. crassa* are the smallest and about one third the length of those from *H. hypogaea*. No effort was made to determine if there are differences in the number of paraphyses per unit area among these species.

All antheridia have a single layer of jacket cells at the gametophyte surface and one opercular cell. Also, the shape of the gametophyte masses for all the species was ellipsoidal. The average size of these masses varies among the species. The gamete mass of *H. cumingii* is larger than those of the other species.

There are variations in the archegonial lengths from egg base to neck tip and the length of the neck protrusion at the gametophyte surface. Variations also occur in the number of neck cell tiers and the number of neck canal cells above the egg. The archegonium of *H. cumingii* with the greatest length from egg base to neck tip does not have the longest neck protrusion. This condition has been noted in comparative studies of other *Lycopodium* (*s. l.*) species (Whittier, unpub.) and it can occur when the egg of a species is sunken more deeply into the gametophyte tissue than in other species.

Prior to the description of the gametophyte of *H. lucidula* (Spessard, 1922), the gametophyte of only one other terrestrial *Huperzia* species was known (Bruchmann, 1898). Bruchmann described a considerable amount of variation with the gametophyte of *Lycopodium selago* (=*H. selago*). He found compact roundish shapes, elongated variously curved axial forms and a range of intermediates. He considered that some variations were caused by the soil conditions. The above variations were found in dense soil and another form was found in loose soil. He showed that young, immature conical gametophytes shifted to dorsiventral, axial gametophytes in loose soil. The dorsiventral, axial gametophytes had paraphyses and gametangia on their dorsal surface and rhizoids ventrally. These dorsiventral gametophytes have the same structure as described for gametophytes of *H. lucidula* (Spessard, 1922).

The dorsiventral axial gametophytes of *H. selago* (Bruchmann, 1898) and *H. lucidula* (Spessard, 1922; Bruce and Beitel, 1979) are Type III gametophytes as recognized by Bruchmann (1898, 1910) for *Lycopodium* (*s.l.*). The dorsiventral forms agree with what has been grown with *H. crassa*, *H. cumingii*, *H. hypogaea* and *H. saururus* in this study. The surface of the semisolid agar of the nutrient medium on which these tropical, terrestrial gametophytes grew is more similar to loose soil than dense soil. The dorsiventral form of the Type III gametophyte appears typical for the terrestrial gametophytes of *Huperzia* on a nutrient medium in culture. Also, the gametophytes of all six terrestrial *Huperzia* species, whether from tropical or temperate regions, that have been described from soil or culture are Type III gametophytes.

Acknowledgments

I acknowledge the assistance of James Hickey with early attempts at growing *Huperzia* gametophytes from the tropics. I thank John Braggins and Benjamin Øllgaard for the spores from Kenya and Ecuador respectively. I am grateful to Larry Peterson for the use of his laboratory facilities in the Department of Botany at the University of Guelph (Canada) where the scanning

electron microscopy was done. I am indebted to Hans-Willi Honegger for help translating sections of Bruchmann's publications.

Literature Cited

Bruce, J. G. and J. M. Beitel. 1979. A community of *Lycopodium* gametophytes in Michigan. Amer. Fern J. 69:33–41.

Bruchmann, H. 1898. *Über die Prothallien und die Keimpflanzen mehrer europäischer Lycopodien.* Friedrich Andreas Perthes, Gotha.

Bruchmann, H. 1910. Die keimung der Sporen und die Entwicklung der Prothallien von *Lycopodium clavatum* L., *L. annotinum* L., und *L. selago* L. Flora 101:220–267.

Johansen, D. A. 1940. *Plant Microtechnique.* McGraw-Hill, New York.

Øllgaard, B. 1987. A revised classification of the Lycopodiaceae *s. lat.* Opera Bot. 92:153–178.

Øllgaard, B. 1989. Index of the Lycopodiaceae. Biol. Skr. 34:1–135.

Spessard, E. A. 1922. Prothallia of *Lycopodium* in America II. *L. lucidulum* and *L. obscurum* var. *dendroideum.* Bot. Gaz. (Crawfordsville) 74:392–413.

Whittier, D. P. 1977. Gametophytes of *Lycopodium obscurum* as grown in axenic culture. Canad. J. Bot. 55:563–567.

Whittier, D. P. 1981. Gametophytes of *Lycopodium digitatum* (formerly *L. complanatum* var. *flabelliforme*) as grown in axenic culture. Bot. Gaz. (Crawfordsville) 142:519–524.

Whittier, D. P. 1998. Germination of spores of the Lycopodiaceae in axenic culture. Amer. Fern J. 88:106–113.

Whittier, D. P. and T. R. Webster. 1986. Gametophytes of *Lycopodium lucidulum* from axenic culture. Amer. Fern J. 76:48–55.

American Fern Journal 96(2):62–65 (2006)

Shorter Notes

The New Flavone Ester Apigenin 7-*O*-*p*-hydroxybenzoate and Three Di-*C*-Glycosylflavones from *Pteris vittata*.—It has been shown by Ma *et al.* (Nature 409:579–580, 2001) that *Pteris vittata* L. is efficient in extracting arsenic from soils; these workers have shown that in fronds of this fern (growing in soil spiked with 1,500 ppm arsenic) arsenic concentrations increased from 29.4 to 15,861 ppm in two weeks whereas arsenic concentration in roots was less than 303 ppm. This is the first report of arsenic hyperaccumulation by an unmanipulated plant. Hence, *Pteris vittata* may be of great interest for phytoremediation of arsenic-contaminated soils that are among the major arsenic sources for drinking water. In addition, Ma *et al.* suggested that these data might be of interest in research dealing with arsenic speciation and distribution in plants. Previous work on the flavonoids of *Pteris vittata* has led to the identification of luteolidin 5-*O*-glucoside by Harborne (Phytochemistry 5:589–600, 1966). In addition, acid hydrolysis of extracts of this fern has led to the identification of kaempferol, quercetin, leucocyanidin and leucodelphinidin by Voirin (Ph. D. thesis, University of Lyon, p. 151, 1970). More recently, 3-*C*-(6″-acetyl-β-cellobiosyl)-apigenin (Amer. Fern J. 89:217–220, 1999) and 6-*C*-β-cellobiosyl-isoscutellarein-8-methyl ether together with quercetin 3-*O*-glucuronide and rutin (Amer. Fern J. 90:42–45, 2000) have been identified by Imperato and Telesca. In addition, three kaempferol glycosides (3-*O*-glucoside, 3-*O*-glucuronide and 3-*O*-(X″-X″-di-protocatechuoyl)-glucuronide), two di-*C*-glycosylflavones (3,8-di-*C*-arabinosyllutelin and 6-*C*-arabinosyl-8-*C*-glucosyl-luteolin), three flavonol glycosides acylated with hydroxycinnamic acids (kaempferol and quercetin 3-*O*-(2″,3″-di-*O*-*p*-coumaroyl)-glucosides together with kaempferol 3-*O*-(X″-*O*-*p*-coumaroyl-X″-*O*-feruloyl)-glucoside), vitexin 7-*O*-rhamnoside and kaempferol 3-*O*-rutinoside have been found by Imperato (Amer. Fern J. 90:141–144, 2000; Amer. Fern J. 92:244–246, 2002; Amer. Fern J. 93:157–160, 2003; Amer. Fern J. 94:159–162, 2004).

In the present paper four flavonoids (I–IV) have been isolated from aerial parts of *Pteris vittata* collected in the Botanic Garden of the University of Naples. The fern has been identified by Dr. R. Nazzaro (University of Naples); a voucher specimen (*149.001.001.01*) has been deposited in the Herbarium Neapolitanum (NAP) of the University of Naples.

Flavonoids I–IV were isolated from an ethanolic extract of aerial parts of *Pteris vittata* by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% HOAc (acetic acid) and BEW (*n*-butanol-ethanol-water, 4:1:2.). Further purification was carried out by Sephadex LH-20 column chromatography eluting with methanol.

Color reactions (brown to yellow in UV+NH_3), chromatographic behaviour (R_f values on Watman No 1 paper: 0.74 in BAW; 0.43 in 15% HOAc) and ultraviolet spectral analysis in the presence of usual shift reagents (λ_{max} (nm)

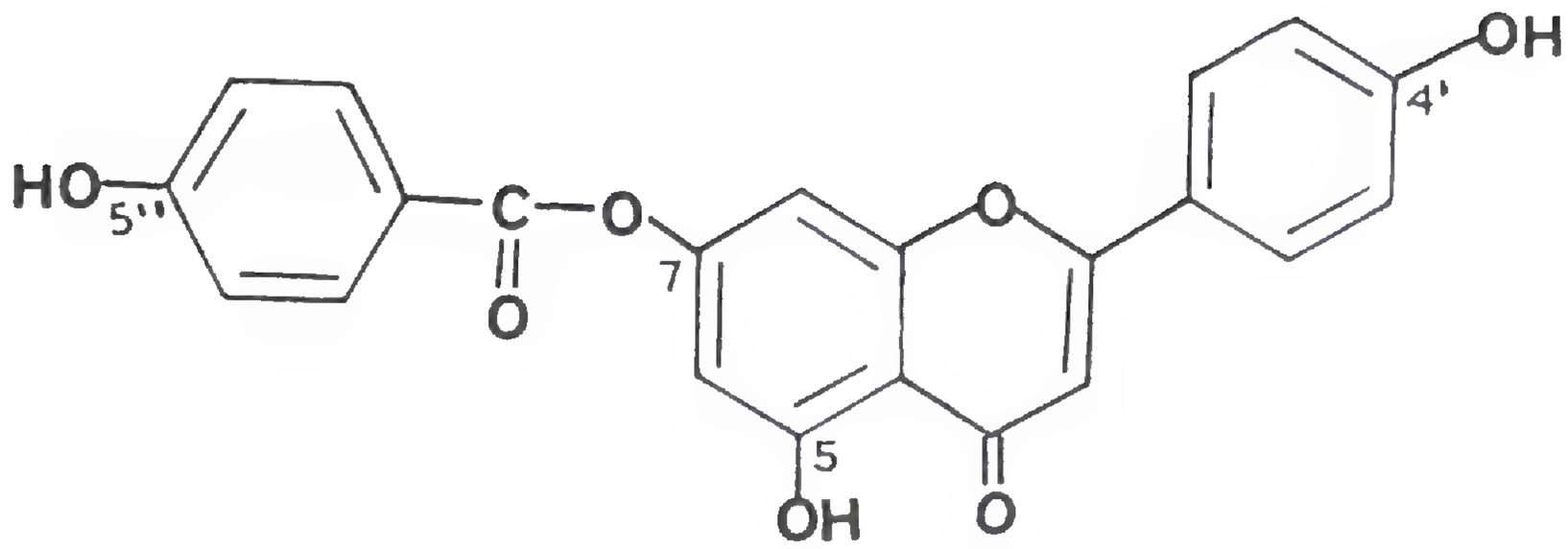


FIG. 1. Structure of Flavonoid I.

(MeOH) 272, 334; +$AlCl_3$ 276, 385; +$AlCl_3$/HCl 277, 384; +NaOAc 272, 382; +NaOMe 273, 383) suggested that flavonoid I may be a flavone with free hydroxyl groups at positions 5 and 4′.

Electrospray mass spectrum showed a pseudomolecular ion at m/z 413 $[M+Na]^+$ suggesting that flavonoid I may be a flavone aglycone esterified with benzoic acid or a hydroxybenzoic acid. Acid hydrolysis (2N HCl; 2h under reflux) gave *p*-hydroxybenzoic acid and apigenin. These data show that flavonoid I is apigenin 7-*O*-*p*-hydroxybenzoate (Fig. 1), a new natural product. The structure of this compound was confirmed by ^{1}H NMR spectrum (300 MHz; DMSO-d_6) which showed signals of apigenin at δ 6.03 (1H, doublet, J = 2 Hz; H-6), δ 6.24 (1H, doublet, J = 2 Hz; H-8), δ 6.71 (1H, singlet; H-3), δ 6.94 (2H, doublet, J = 8.6 Hz; H-3′ and H-5′), δ 7.98 (2H, doublet, J = 8.6 Hz; H-2′ and H-6′); signals of *p*-hydroxybenzoyl group appeared at δ 6.82 (2H, doublet, J = 8.3 Hz; H-4″ and H-6″) and δ 7.61 (2H, doublet, J = 8.3 Hz; H-3″ and H-7″).

This is the first report of the occurrence of a flavone aglycone acylated with an aromatic acid in ferns. A single ester of a flavonoid aglycone with an aromatic acid has previously been reported from plants; this compound was isolated from leaf resin of *Baccaris bigelovii* A. Gray (Asteraceae) and has been identified as chrysin 7-*O*-benzoate by Arriaga-Giner *et al.* (Z. Naturforsch 41c:946–948. 1986). Thirteen methylated flavonol aglycones, acylated with aliphatic acids (acetic and butyric) have previously been found in ferns belonging to the genus *Notholaena* as shown in the review of Wollenweber (pp. 259–335, *in* J. B. Harborne, ed., *The Flavonoids, Advances in Research Since 1986*, Chapman and Hall, London, 1994). In flavonoid glycosides *p*-hydroxybenzoic has been found as acyl substituent in kaempferol 3-(*p*-hydroxybenzoylglucoside)-7-glucoside from *Narcissus poeticus* L. (Amaryllidaceae) by Schoensiegel *et al.* (Z. Naturforsch 24 b:1213–1214, 1969) and in quercetin 3-α-(2″-*p*-hydroxybenzoyl-4″-*p*-coumaroylrhamnoside) from *Libocedrus bidwillii* Hook. f. (Cupressaceae) by Franke and Markham (Phytochemistry 28:3565–3567, 1989).

R_f values on Whatman No 1 paper, color reactions and UV spectral analysis in the presence of usual shift reagents suggested that flavonoid II may be a flavone glycoside with free hydroxyl groups at positions 5, 7, 3′ and 4′. This product may be a *C*-glycosylflavone since it was resistant to acid hydrolysis

(2N HCl; 2 hr under reflux). Electrospray mass spectrum showed a pseudomolecular ion at m/z 603 $[M+Na]^+$, an ion at m/z 1183 $[(Mx2)+Na]^+$ (dimer), an ion at m/z 1809 $[(Mx3)+3\ Na]^+$ (trimer) and a fragment ion at m/z 441 [*C*-pentosylluteolin+Na]$^+$; these data show that Flavonoid II is a *C*-exosyl-*C*-pentosylluteolin. Since Wessely-Moser isomerization (3N HCl; 3h under reflux) gave 6-*C*-arabinosyl-8-*C*-glucosylluteolin (previously found in *Pteris vittata* by Imperato (Amer. Fern J. 92:244–246, 2002)), Flavonoid II is identified as 6-*C*-glucosyl-8-*C*-arabinosylluteolin (carlinoside) which is a new fern constituent. Identification was confirmed by ^{1}H NMR spectrum and ^{13}C NMR spectrum.

Flavonoid III was identified as schaftoside (6-*C*-glucosyl-*8*-*C*-arabinosylapigenin) by UV spectral analysis in the presence of usual shift reagents, R_f values, color reactions, electrospray mass spectrum (which showed a pseudomolecular ion at m/z 587 $[M+Na]^+$ (*C*-exosyl-*C*-pentosylapigenin) and an ion at m/z 1151 $[Mx2+Na]^+$ (dimer)), ^{1}H NMR spectrum and ^{13}C NMR spectrum. Schaftoside has previously been found in *Angiopteris lygodiifolia* Ros. (Mariattaceae) by Wallace *et al.* (Phytochemistry 20:2701–2703, 1981), in *Polypodium vulgare* L. (Polypodiaceae) by Karl *et al.* (Z. Naturforsh 37c:148–153, 1982) and in *Archangiopteris henryi* Christ & Gies. (Angiopteridaceae) by Wallace (Biochem. Syst. Ecol. 15:529–530, 1987).

Flavonoid IV was present in trace amounts and was resistant to acid hydrolysis (2N HCl; 2h under reflux). It was partially characterized as di-*C*-pentosylapigenin by UV spectral analysis with usual shift reagents and electrospray mass spectrum which showed a pseudomolecular ion at m/z 557 $[M+Na]^+$ (di-*C*-pentosylapigenin) and an ion at m/z 1091 $[M\times2+Na]^+$ (dimer).

The presence of three dimers and one trimer in electrospray mass spectra of di-*C*-glycosylflavones II–IV may be due to hydrogen bonding in gas phase. Hydrogen bonding in crystal structures of flavonoids has been shown by a number of authors, e.g. by Cantrell (pp. 391–394, *in* V. Cody, E. Middleton, Jr. and J. B. Harborne, eds., *Plant Flavonoids in Biology and Medicine*, Alan R. Liss, New York, 1986).

The presence of *C*-glycosylflavones II–IV in *Pteris vittata* and the four *C*-glycosylflavones previously reported from this fern show that *Pteris vittata* has an impressive range of *C*-glycosylflavones; a similar situation has been observed in *Angiopteris evecta* (G. Forst.) Hoffm. (Mariattaceae) as shown in the review of Markham (pp. 427–468, *in* J. B. Harborne, ed., *The Flavonoids: Advances in Research since 1980;* Chapman and Hall, London, New York, 1988) but this fern lacks flavonoid *O*-glycosides which are present in *Pteris vittata*.

Polyphenols of ferns have been investigated by Imperato and co-workers since 1978. It is well known that flavonoids are of interest in chemotaxonomic and phylogenetic studies but flavonoid data are scanty for many fern families. Ninety flavonoids including sixty-three new fern constituents have been isolated from ferns in this laboratory as shown in the review by Markham (1988), in the review by Imperato (pp. 39–75 *in Current Topics in Phytochemistry*, Research Trends, Trivandrum, 2000) and in the introduction of this paper; these flavonoids include fifty new natural products. Among the

new compounds, kaempferol 3-*O*-(X″-*p*-coumaroyl-X″-heptaglycylapioside) isolated by Imperato (Amer. Fern J. 86:127–128, 1996) from *Pteridium aquilinum* is the only known flavonoid occurring in such bound form in plants. The isolation of bracteatin by Imperato (La Chimica & L'Industria 71:86–87, 1989) from *Asplenium kaulfussii* Schltdl. represents the only known report of an aurone from ferns. 3-*C*-Xylosylapigenin 7-methyl ether and 3-*C*-rhamnosylluteolin isolated by Imperato (Phytochemistry (Life Science Advances) 11:225–230, 1992) from *Asplenium viviparum* belong to a new type of mono-*C*-glycosylflavones; in addition the isolation by Imperato (Phytochemistry 33:729–730, 1993) of 3,6,8-tri-*C*-xylosylapigenin from *Asplenium viviparum* (L.f.) Pr. represents the first report of a tri-*C*-glycosylflavonoid from plants. It is well known that Smith and Levin (Am. J. Bot. 50:952–958, 1963) provided a clear example of additive inheritance of chemical characters in *Asplenium* hybrids; subsequently Richardson and Lorenz-Liburnau showed (Amer. Fern J. 72:103–106, 1982) that the chemistry of European *Asplenium adiantum-nigrum* L. complex is analogous to that of Appalachian *Asplenium* complex. Thirty-two flavonoid glycosides from *Asplenium* species have been fully characterized in this laboratory as shown in two reviews by Imperato (Biochem. Syst. Ecol. 17:161–166, 1989 and the above review (2000)).

In 1961 Harborne and Corner (Biochem. J. 81:242–250, 1961) showed that hydroxycinnamic acid-sugar derivatives are of wide occurrence in higher plants; subsequently three papers by Bohm and Tyron (Can. J. Bot. 45:585–593, 1967), by Bohm (Phytochemistry 7:1825–1830, 1968) and by Glass and Bohm (Phytochemistry 8:629–632, 1969) showed that hydroxycinnamic acids are widespread in ferns with the exception of sinapic acid. Seventeen hydroxycinnamic acid-sugar derivatives including nine new natural products have been isolated by Imperato from ferns belonging to the genera *Asplenium*, *Ceterach*, *Adiantum* and *Cystopteris* as shown in the review by Imperato (2000). Most of these compounds are 1-*p*-coumaroylglucose sulphates and 1-caffeoylglucose sulphates having sulphate attached to an hydroxyl group of sugar moiety; in two of these products glucose is replaced by galactose or by a disaccharide (laminaribiose).

As shown in the review by Richardson (Biochem Syst. Ecol. 12:1–6, 1984) xanthones of ferns may confirm or reveal the relationships between diploids and allopolyploids, although these compounds are of little interest in fern taxonomy. Fourteen xanthones, including nine new natural products have been found in ferns in this laboratory; among these products 3,7,8-trihydroxy-1-*O*-β-laminaribioside isolated by Imperato from *Asplenium adiantum-nigrum* (Phytochemistry 19:2030–2031,1980) is the first xanthone glycoside having the carbohydrate moiety attached to a phenolic hydroxyl group. In addition, 1,6-dihydroxy-3,5,7-trimethoxyxanthone isolated by Imperato from *Cystopteris fragilis* (J. Nat. Prod. 54:603–604, 1991) is the first pentaoxygenated xanthone found in ferns.

The author thanks Università della Basilicata for financial support. Mass spectra were provided by SESMA (CNR, Naples).—Filippo Imperato, Dipartimento di Chimica, Università della Basilicata, 85100 Potenza, ITALY.

American Fern Journal 96(2):66 (2006)

Review

Flora de la República de Cuba: Serie A. Plantas Vasculares. Fascículo 11. 2006. Koeltz Scientific Books, Koenigstein, Germany. 236 pp. Paperback [ISBN 3-906166-45-7/978-3-906166-45-2] Euro 56.00.

This fascicle is the third published on ferns and lycophytes in the Cuban flora series. The other families previously published were the Hymenophyllaceae (fascicle 4), and the Aspleniaceae and Cyatheaceae (fascicle 8, along with Cycadaceae and Zamiaceae). The present fascicle treats 13 families: Azollaceae, Dicksoniaceae, Equisetaceae, Isoetaceae, Lophosoriaceae, Marsileaceae, Oleandraceae, Ophioglossaceae, Osmundaceae, Plagiogyriaceae, Psilotaceae, Salviniaceae, and Thelypteridaceae. These contain a total of 15 genera and 88 species. By far the most important family is the Thelypteridaceae, which contains 65 species, 14 of which (21.5%) are endemic. The family treatments were co-authored by the following pteridologists, in varying combinations: Manuel G. Caluff, James Hickey, David M. Johnson, Ramona Oviedo, Mónica Palcios-Rios, Gustavo Shelton, Ledis Regalado, and Carlos Sánchez.

The keys are of the non-indented type, and illustrations are generally limited to one per family (the large Thelypteridaceae has more). An index to scientific names is given after each family treatment, but there is no cumulative index at the end of the entire fascicle. Dot-distribution maps are given, and these are attractive, although the dots are a bit on the small side. A novel aspect of this flora is that the dot maps and specimen data upon which they are based are included on a CD.

It is hoped that remaining fascicles can appear in the next decade or so. The completion of a Cuban fern and lycophyte flora would be a most welcome contribution to pteridology.—Robbin C. Moran, The New York Botanical Garden, Bronx, NY 10458-5126.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerox copies acceptable), including review copies of illustrations and originals of illustrations. After review, submission of final versions of manuscripts on diskette (in PC- or Mac-compatible formats) is strongly encouraged. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, short, informative abstract, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Do not submit right-justified copy, avoid footnotes, and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence et al., 1968, Hunt Botanical Library, Pittsburgh) and its supplement (1991). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, omit the abstract and put all references parenthetically in text. Use *Index Herbariorum* (Regnum Veg. 120:1–693. 1990) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matter of form or style, consult recent issues of *American Fern Journal* and *The Chicago Manual of Style*, 14th ed. (1993, Univ. Chicago Press, Chicago). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

PTERIDOLOGIA ISSUES IN PRINT

The following issues of *Pteridologia*, the memoir series of the American Fern Society, are available for purchase:

1. Wagner, David H. 1979. Systematics of Polystichum in Western North America North of Mexico. 64 pp. $10.00 postpaid.

2A. Lellinger, David B. 1989. The Ferns and Fern-allies of Costa Rica, Panama, and the Chocó (Part 1: Psilotaceae through Dicksoniaceae). 364 pp. $32.00 postpaid.

3. Lellinger, David B. 2002. A Modern Multilingual Glossary for Taxonomic Pteridology. 263 pp. $28.00 postpaid.

Send your order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

AMERICAN FERN JOURNAL ON MICROFICHE

Volumes 1–61 of the *American Fern Journal* are available as archival quality, silver positive microfiches. Single volumes or the entire run may be purchased. The fiches are easily read with 10x or greater magnification (using a dissecting microscope and transmitted illumination or a fiche reader). Silver negative microfiches of vols. 1–50 are also available. The price is $4.00 per volume or $244.00 per set of 61 volumes, postpaid.

Send your inquiry or order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

FIDDLEHEAD FORUM

The editor of the Bulletin of the American Fern Society welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

SPORE EXCHANGE

Ms. Denia Mandt, 12616 Ibbetson Ave., Downey, CA 90242-5050, is Director. Spores exchanged and lists of available spores sent on request. http://amerfernsoc.org/sporexy.html

GIFTS AND BEQUESTS

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Back issues of the Journal and cash or other gifts are always welcomed and are tax-deductible. Inquiries should be addressed to the Secretary.

VISIT THE AMERICAN FERN SOCIETY'S WORLD WIDE WEB HOMEPAGE: http://www.amerfernsoc.org/

AMERICAN FERN JOURNAL

Volume 96

Number 3

July–September 2006

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Morphological and Anatomical Variation in Sporophylls of *Isoetes sinensis* Palmer (Isoetaceae), an Endangered Quillwort in China
Hong Liu, Qing Feng Wang and W. Carl Taylor **67**

***Blechnum ludificans* Herter, an Overlooked Fern from South America**
E. R. de la Sota, G. E. Giudice and M. L. Luna **75**

A New Species of *Microlepia* (Dennstaedtiaceae) from Mt. Micangshan, China
Xiao-si Guo and Bin Li **80**

Fern Laminar Scales Protect Against Photoinhibition from Excess Light
J. E. Watkins, Jr., Akito Y. Kawahara, Stacy A. Leicht, Josh R. Auld, Abram J. Bicksler and Kristine Kaiser **83**

SHORTER NOTES

Kaempferol 3-*O*-(acetylrutinoside), a New Flavonoid and Two New Fern Constituents, Quercitin 3-*O*-(acetylglucoside) and 3-*O*-(acetylrutinoside) from *Dryopteris villarii*
Filippo Imperato **93**

***Deparia longipes* (Woodsiaceae) Native to Taiwan**
Wataru Shinohara, Tsai-Wen Hsu, Shann-Jye Moore, Ho-Ming Chang and Noriaki Murakami **96**

REVIEW

Ferns of Northeastern and Central North America, 2nd ed. *R. James Hickey* **100**

The American Fern Society

Council for 2006

DAVID S. CONANT, Dept. of Natural Sciences, Lyndon State College, Lyndonville, VT 05851.
President

WARREN D. HOAK, Dept. of Biological Sciences, Denison University, Granville, OH 43023
President-Elect

W. CARL TAYLOR, 800 W. Wells St., Milwaukee Public Museum, Milwaukee, WI 53233-1478.
Secretary

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916-1110.
Treasurer

GEORGE YATSKIEVYCH, Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299.
Membership Secretary

JAMES D. MONTGOMERY, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.
Curator of Publications

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Journal Editor

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Memoir Editor

ROBIN HALLEY, 1418 Park Row, La Jolla, CA 92037-3710
DAVID SCHWARTZ, 9715 Christey Way, Bakersfield, CA 93312-5617
Bulletin Editors

American Fern Journal

EDITOR

R. JAMES HICKEY ..... Botany Department, Miami University, Oxford, OH 45056,
ph. (513) 529-6000, e-mail: hickeyrj@muohio.edu

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47405-6801
GARY K. GREER ..... Biology Dept., Grand Valley State University, Allendale, MI 49401
CHRISTOPHER H. HAUFLER ..... Dept. of Ecology and Evolutionary Biology, University of Kansas,
Lawrence, KS 66045-2106
ROBBIN C. MORAN ..... New York Botanical Garden, Bronx, NY 10458-5126
JAMES H. PECK ..... Dept. of Biology, University of Arkansas–Little Rock,
2801 S. University Ave., Little Rock, AR 72204

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at The American Fern Society, % Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299. Periodicals postage paid at St. Louis, MO, and additional entry.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.

Changes of address, dues, and applications for membership should be sent to the Membership Secretary.

General inquiries concerning ferns should be addressed to the Secretary.

Back volumes are avalible for most years as printed issues or on microfiche. Please contact the Back Issues Curator for prices and availability.

Subscriptions:

Society Membership – USA, Canada, Mexico (includes Journal and Fiddlehead Forum)	$25
Society Membership – All other countries (includes Journal and Fiddlehead Forum)	$32
Society Life Membership – $300 (add $140 mailing surcharge for outside USA, Canada, Mexico)	
Regular Membership – USA, Canada, Mexico (includes Fiddlehead Forum)	$12
Regular Membership – All other countries (includes Fiddlehead Forum)	$19
Institutional Membership – $35 to USA, Canada, Mexico; $45 elsewhere (-$2 agency fee)	

POSTMASTER: Send address changes to American Fern Journal, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

American Fern Journal 96(3):67–74 (2006)

Morphological and Anatomical Variation in Sporophylls of *Isoetes sinensis* Palmer (Isoetaceae), an Endangered Quillwort in China

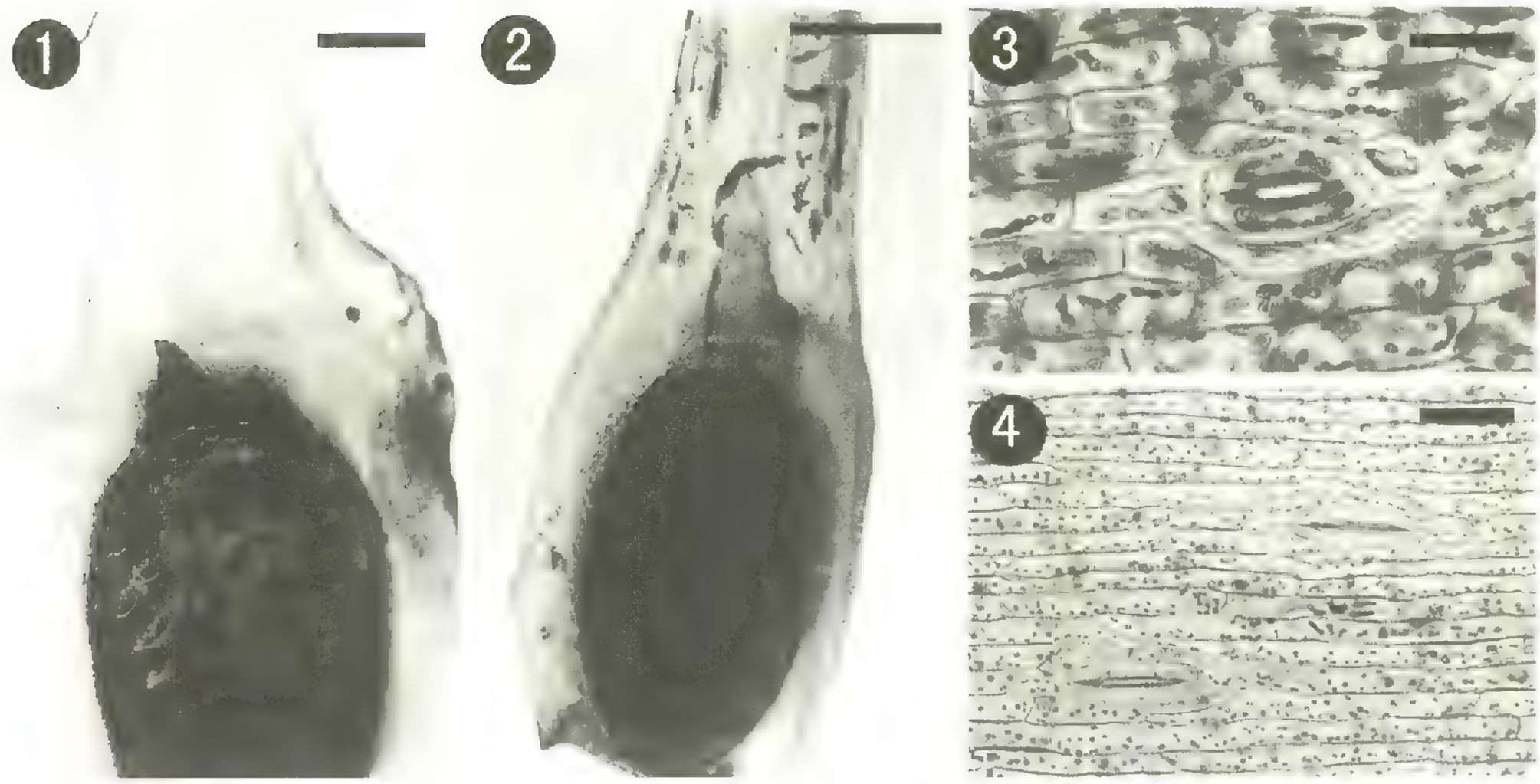
HONG LIU
College of Life Sciences, South-Central University for Nationalities, Wuhan, 430074, P. R. China
QING FENG WANG[1]
Laboratory of Plant Systematics and Evolutionary Biology, College of Life Sciences, Wuhan University, Wuhan, 430072, P. R. China.
W. CARL TAYLOR
Department of Biology, Old Dominion University, Norfolk, VA 23529

ABSTRACT.—*Isoetes sinensis* is a rare and endangered plant in wetlands of southeastern China. Previous studies have reported the chromosome number, geographic distribution, and ecology of this Asian endemic, but there has not been an analysis of diagnostic characters associated with the sporophylls of *I. sinensis* in China. Therefore, morphological and anatomical variation of sporophylls in the three known Chinese populations of *I. sinensis* were evaluated and compared. The variation found is discussed in relation to the present taxonomy of *I. sinensis*.

Isoetes L. is a cosmopolitan genus with an estimated 150 extant species (Tryon and Tryon, 1982; Talyor and Hickey, 1992). Five species of *Isoetes* are known to occur in China. These include three basic diploids ($2n = 22$): *I. yunguiensis* Wang Q. F. & W. C. Taylor, *I. hypsophila* Handel-Mazzetti and *I. taiwanensis* DeVol, one tetraploid ($2n = 44$) *I. sinensis* Palmer, and one hexaploid ($2n = 66$) *I. orientalis* H. Liu & Q. F. Wang (Liu *et al.*, 2002; Wang *et al.*, 2002; Liu *et al.*, 2005). Palmer's (1927) original description of *Isoetes sinensis* was based on specimens from Nanjing, China, from which he described the megaspores as "thickly set with high, stout, blunt or crested columns, more or less confluent into crested ridges especially below" and the microspores as "densely spinulose." *I. sinensis* has been reported from China, Japan, and Korea (Takamiya *et al.*, 1997; Takamiya, 2001). The tetraploid cytotype of *I. sinensis* was first reported in China by He *et al.* (2002). A hexaploid taxon, found in Korea and Japan, was originally described as *I. coreana* by Chung and Choi (1986), but Takamiya *et al.* (1997) treated this cytotype as *Isoetes sinensis* var. *coreana* (Y. H. Chung & H. K. Choi) M Takamiya, M. Watanabe & K. Ono.

Isoetes sinensis is an aquatic fern confined to clear waters (Pang *et al.*, 2003). In recent years, it has decreased in numbers and even disappeared from several locations in mainland China (Foster *et al.*, 1974). In our recent field investigation, only three populations remained in mainland China. It is now considered to be rare and endangered in China and it is listed as a first category protected wild plant species (Yu, 1999).

[1] Author for Correspondence.



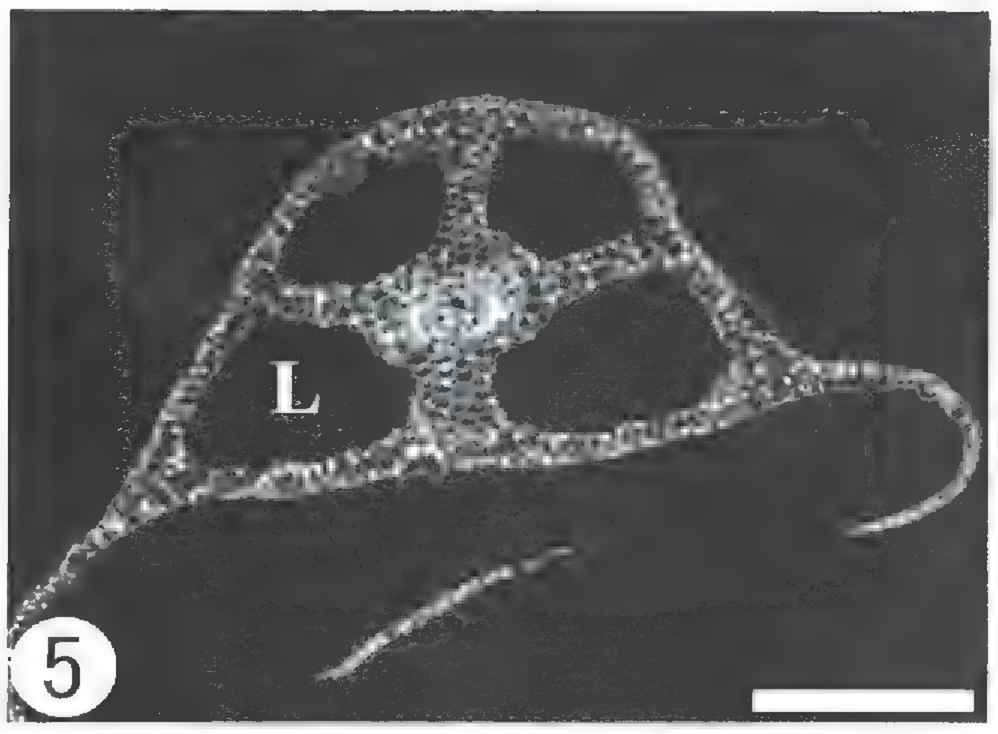
FIGS. 1–4. Foliar characters of *I. sinensis*. 1. Leaf base of megasporophyll showing small, deltate ligule (pop. JD 1). 2. Leaf base of megasporophyll showing long triangular ligule (pop. XN 1). 3. Close up of epidermis and stomatal apparatus (pop. JD 1). 4. Epidermis and stomatal apparatus (pop. XL 1). Bars in 1, 2 = 4 mm; bars in 3, 4 = 40 μm.

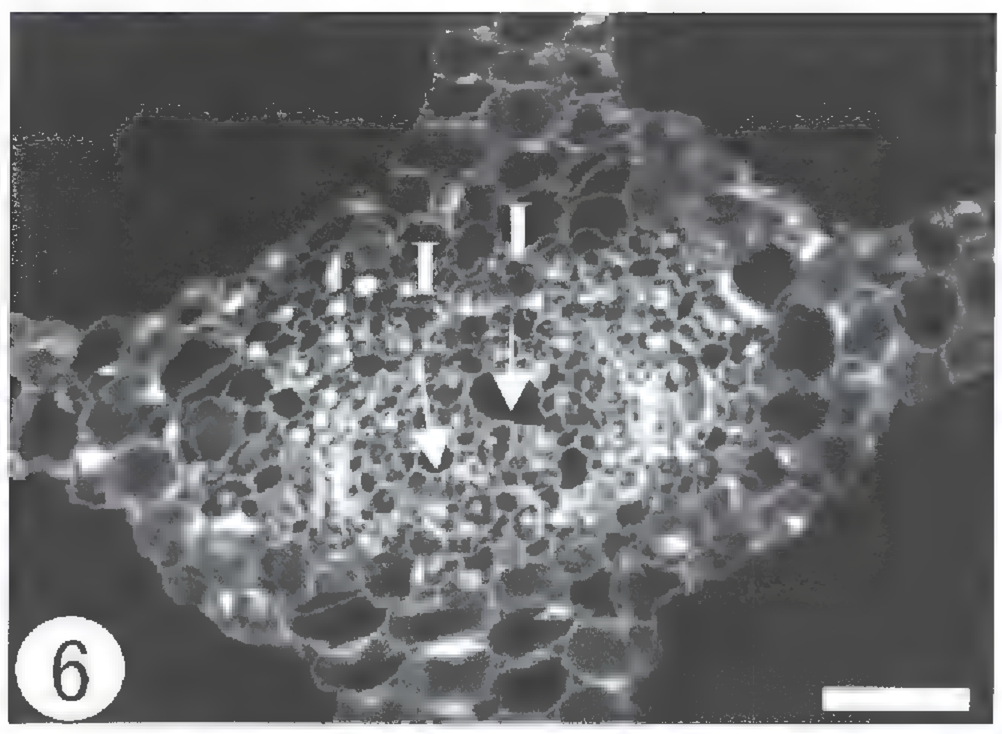
Plants of *I. sinensis* have linear leaves arising spirally from a depressed shoot apex on an underground, subglobose, 3 or 4-lobed rootstock. Its dichotomous roots arise synchronously from the furrows between the lobes on the undersurface of the rootstock. Leaves of *I. sinensis* are brittle, stiffly erect, and each contains four longitudinal air canals (Figs. 5–8). The length of mature leaves ranges from 15–30 cm. Sporangia develop in a concavity in the expanded base of each leaf (Figs. 1, 2). A deltoid ligule occurs above the concavity. Plants are typically monoecious with external megasporophylls and internal microsporophylls; dioecious plants are rare. Megasporphylls are produced earlier than microsporophylls in the growing season, are peripherial and have megasporangia containing 200–500 globose megaspores averaging about 450 μm in diameter. Microsporophylls are produced later in the growing season are more central and each has a microsporangium containing thousands of ellipsoid microspores averaging about 30 μm in length.

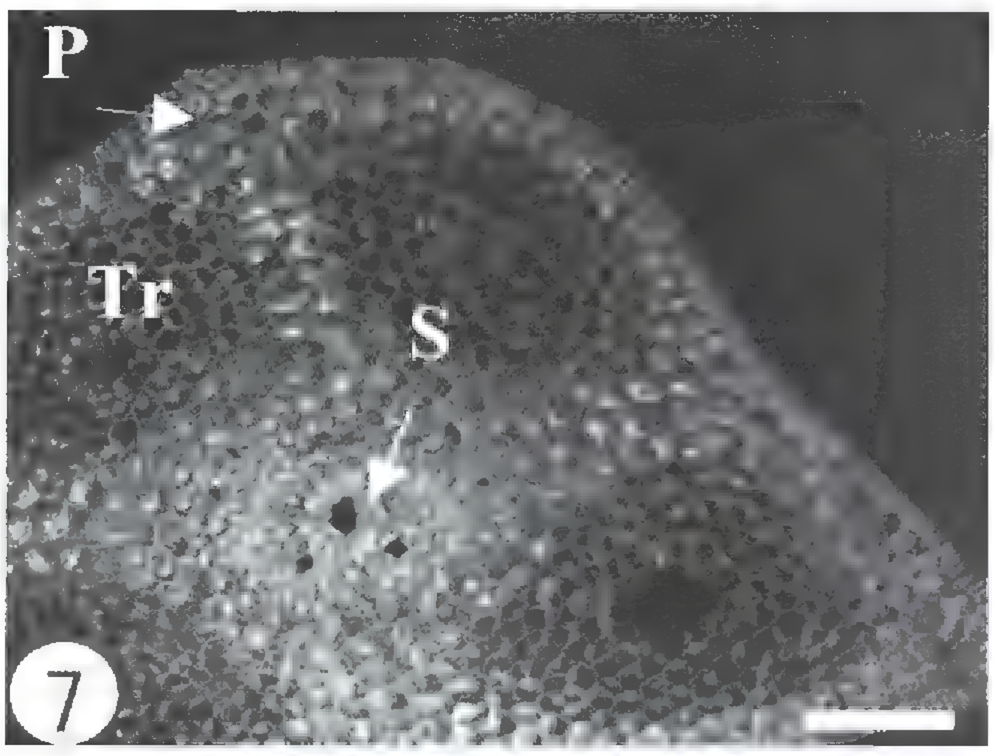
The goals of this study were to document morphological and anatomical variation in sporophylls across and within three populations of *I. sinensis* in China and evaluate how this variation relates to the present taxonomy of *I. sinensis*.

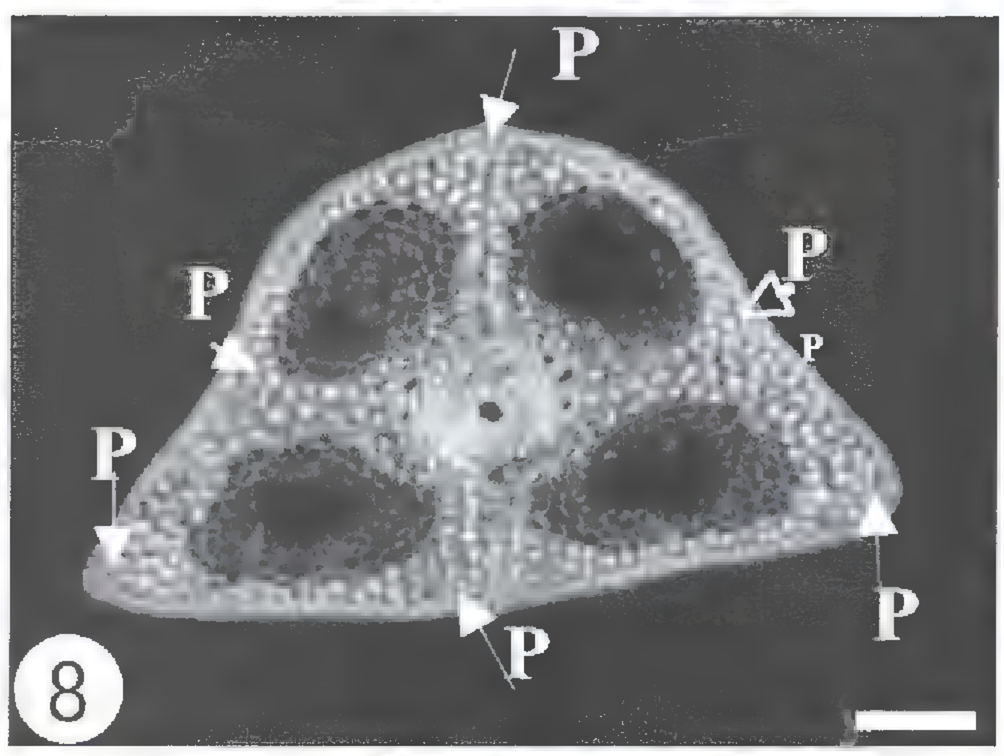
MATERIALS AND METHODS

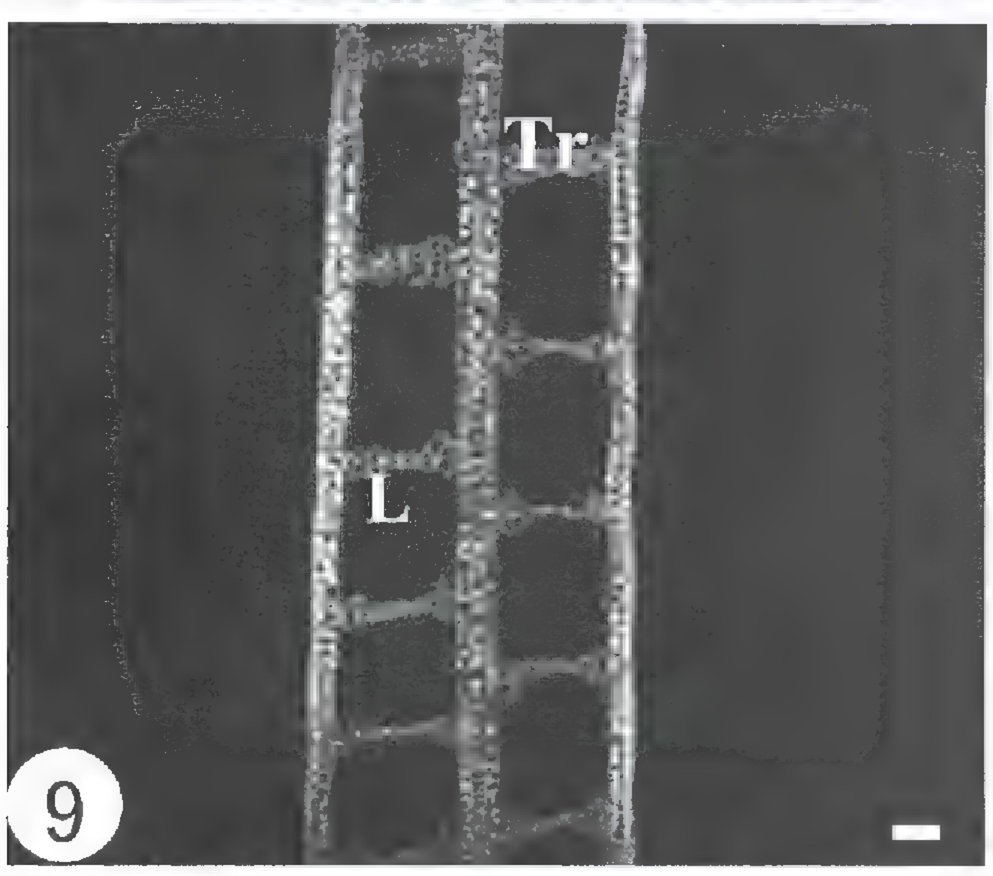
Three populations of *Isoetes sinensis* were identified in southeastern China. Population XN1 lies in a marshy and abandoned field at Xiuning, Anhui Province at an elevation of 360 m. Populations JD1 and JD2 are in Jiande, Zhejiang Province at an elevation of 134 m. The latter two populations occur

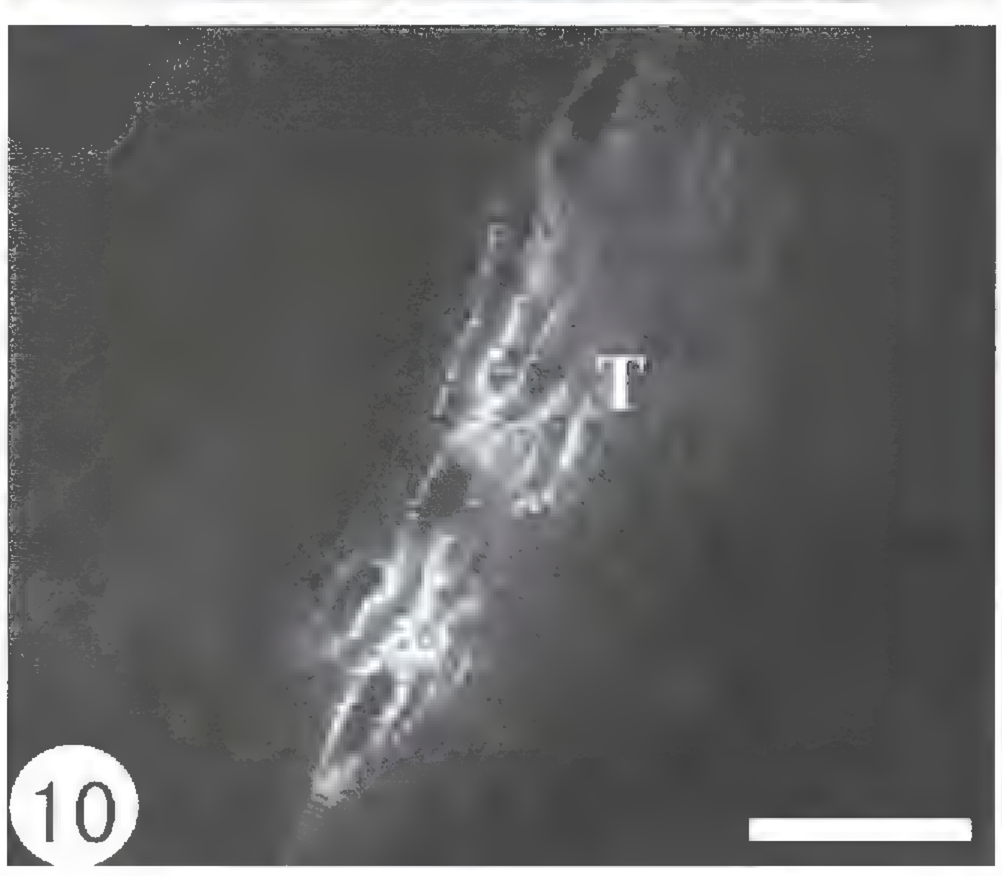












FIGS. 5–10. Anatomical characters of *I. sinensis*. 5. Proximal leaf transverse section showing four air chambers (pop. JD 1). 6. Close up of Fig 3 showing three intrastelar canals (pop. JD 1). 7. Distal leaf transverse section showing three intrastelar canals and leaf trabeculae (pop. JD 2). 8. Distal leaf transverse section showing four air chambers and one intrastelar canal (pop. XN 1). 9. Longitudinal leaf section showing air chambers and diaphragms, uniform within populations JD 1, JD 2 and XN 1. 10. Longitudinal section showing annular tracheids. Bar in 5 = 800 μm; Bars in 6–9 = 200 μm; Bar in 10 = 75 μm; I = intrastelar canal; L = lacuna; P = peripheral fiber strand; S = stele; T = tracheid; Tr = trabeculae.

in the littoral zone of Xingan-jiang River where, due to an upstream hydroelectric dam, water level changes diurnally to completely expose plants for several hours each day. JD1 was separated from JD2 by a one kilometer stretch of coast along the river. Mature plants were collected from each of the three populations in December, 2002. Each plant was labeled and cultivated in a shallow pot in the Wuhan University Botanical Garden.

Morphological features of sporophylls, including number and length of sporophylls, ligule shape, velum, megaspore number per megasporangium, spore size, and guard cell length, were recorded from fresh, field-collected specimens and from specimens fixed in FAA. Anatomical features of sporophylls were observed in cultivated plants from hand sections of fresh samples and microtome sections of fixed samples. A Leica DMIRE2 microscope fitted with a MicroMax CCD Camera employing MetaMorph software (Universal Imaging Corporation, series 4.0) were used to capture images. Microscopic measurements were made using an ocular micrometer.

Sporophyll number was counted and sporophyll length was measured for twenty-five plants randomly selected from each of the three populations. Ligule shape was evaluated on each of these sporophylls. To gauge stomata guard cell length, the leaf epidermis was peeled from five plants in each population and the length of twenty-five guard cells from each plant was measured. To establish the average number of megaspores per megasporangium, the number of megaspores in 200 megasporangia from each population, were counted. For spore size, one mature megasporangium and microsporangium were taken from each of five randomly chosen individuals in each population. Twenty air dried megaspores and twenty re-hydrated microspores were measured from each sporangium for a total sample size of 100 megaspores and 100 microspores from each population. Leaf sections were made just above the insertion of the ligule and at the mid-length of the leaf.

Specimens for microtome sectioning were harvested, fixed in FAA, and after passing through an alcohol dehydration series were embedded in paraffin (Johanson, 1940). Microtome sections were cut at 6–8 μm.

Results and Discussion

Morphological features.—Scale leaves were absent and the velum was rudimentary in all the plants sampled from all three populations. The labium in populations JD1 and JD2 was larger than in population XN1. The labium of plants in populations JD1 and JD2 was ca. 680 μm long, whereas the labium in population XN1 was slightly shorter, ca. 600 μm long. The labium width in JD1 and JD2 was ca. 300 μm, whereas labium width in population XN1 was considerably smaller, ca. 130 μm wide. Ligule shape also showed variation. Plants of XN1 had ligules that were long triangular and prominent, but in JD1 and JD2 the ligule was deltate, cordate, shorter, and indistinct (Table 1). Representative ligule shapes of *I. sinensis* from populations JD1 and JD2 are shown in Fig. 1 and from population XN1 in Fig. 2.

TABLE 1. Comparison of different populations *Isoetes sinensis*.

Characters	Population		
	XN1	JD1	JD2
Location	Xiuning, Anhui	Jiande, Zhejiang	Jiande, Zhejiang
Chromosome number	44	44	44
Elevation (m)	360	134	134
Reproduction	sexual	sexual	sexual
Habitat	abandoned rice field (marsh)	fresh water intertidal zone	fresh water intertidal zone
Habit	emergent	submerged	submerged
Scale leaf	absent	absent	absent
Labium	present, tumefy	present, subulate	present, subulate
Velum	absent	absent	absent
Ligule shape	elongated and deltoid	cordate and smallish	cordate and smallish
Corm lobe number	3	3–4	3–4
Leaf number (n = 25)	12.08 ± 7.53	44.60 ± 34.12	13.2 ± 7.46
Leaf length (cm, n = 25)	13.08 ± 4.91	12.54 ± 3.98	7.96 ± 3.32
Peripheral fibres	4	4–6	4
Intrastelar canal number	1	3	3
Guard cell length (μm)	63.1 ± 7.28	65.42 ± 4.76	67.35 ± 5.70
Megaspore number (n = 200)	235.2 ± 87.26	411.6 + 115.45	397.8 ± 166.28
Megaspore ornamentation	cristate	cristate	cristate
Megasopre diameter (μm; n = 100)	448.8 ± 43.91	418.9 ± 31.36	354.3 ± 36.68
Microspore ornamentation	echinate	echinate	echinate
Microspore length (μm; n = 100)	27.13 ± 2.71	34.07 ± 3.21	27.63 ± 3.24

In all three populations investigated, the leaves of *I. sinensis* were light green and erect to stoutly ascending. There was notable variation in leaf length, leaf number and spore size among populations (Table 1). The mean leaf lengths in the three populations were 13.08 ± 4.91 cm (XN1, n = 25), 12.54 ± 3.98 cm (JD1, n = 25), and 7.96 ± 3.32 cm (JD2, n = 25). The mean numbers of leaves per plant in the three populations were 12.08 ± 7.53 (XN1, n = 25), 44.60 ± 34.12 (JD1, n = 25), and 13.2 ± 7.46 (JD2, n = 25). The mean number of megaspores per megasporangium (n = 200) of JD2 is about 411.6, much larger than the 235.2 mean of XN1. Average megaspore size of XN1 is 448.9 ± 43.91 μm, much larger than that of JD2 (354.3 ± 26.67 μm). The average microspore size of JD1 is the largest among the three populations. The size of megaspores and microspores produced by *I. sinensis* is variable and appears to be dependent upon climate and locality. Love (1962) reported that the smallest spores of boreal aquatic *I. echinospora* were found on plants from the deeper

and colder parts of lakes, whereas the spores were invariably larger in shallower and warmer parts of the same lakes. These observations also indicate that local environments can affect *Isoetes* spore size. Cox and Hickey (1984) stated that "Collections of a single species along a transect from shallow to deeper water show a corresponding increase in leaf length" and their analysis of three populations of *I. storkii* found that the population "highest in altitude has the smallest spores". The data from three populations of *I. sinensis* seem fully supports the first statement of Cox and Hickey (1984), but differ from their latter view.

Pfeiffer (1922) considered the presence or absence of stomata to be a reliable taxonomic character in *Isoetes*, whereas Kott and Britton (1985) considered it to be a feature dependent upon growing conditions. The presence of stomata increases photosynthetic potential and safeguards xylem from cavitation (Woodward, 1998). Numerous stomata were observed on the upper one-third of the leaves in all three populations of *I. sinensis* examined. Stomatal guard cell length showed slight variation among the three populations investigated. The guard cell length for JD1 was 65.42 ± 4.76 μm, JD2 was 67.35 ± 5.70 μm and for XN1 was 63.1 ± 7.28 μm.

Anatomical features.—Leaf shape in transverse section at the uppermost part of the membranous leaf margin reveals features common to all *Isoetes*. For example, in Figs. 5 and 8, the four air chambers surrounding the stele can be seen; trabeculae or cross partitions composed of parenchyma (Figs. 7, 9) also characterize the leaves of *Isoetes*.

Within the stele, three small cavities called intrastelar canals were present in JD1 (Figs. 5–6) and JD2 populations (Fig. 7), but only one intrastelar canal was seen in XN1 (Fig. 5). Previous studies revealed that the number of canals appeared to be a stable diagnostic feature for *Isoetes* (Takamiya *et al.*, 1997). Around the intrastelar canal, the annular tracheids can be seen clearly (Fig. 10). The internal leaf anatomy of *Isoetes* shows less environmentally induced variation than the external leaf morphology. Therefore, because of their stability, anatomical characters of leaves have been considered to be of greater diagnostic value (Pfeiffer, 1922).

The peripheral fiber strands (long arrows in Figs. 7 and 8) in *Isoetes* leaves are bundles of thick-walled fibers situated under the epidermis that provide structural support. The presence or absence and the number and position of peripheral fiber strands have been used as diagnostic characters by Pfeiffer (1922) and Wanntorp (1970). West and Takeda (1915) examined the leaf anatomy of *Isoetes* in Japan and found that the peripheral fiber strands were located in four main (one abaxial and three adaxial) bundles and two lateral accessory bundles that were not always continuous along the length of leaves. They also noted that the presence or absence of these strands in the leaves appeared to depend upon the environmental conditions under which the plants grew. In the three populations examined for this study, the peripheral fiber strands were always seen at the four main sites (long arrows in Fig. 8) and the two lateral accessory sites (short arrows in Fig. 8) and the lateral strands were always discontinuous.

Taxonomic Treatment.—Isoetes sinensis, described from China by Palmer (1927), also occurs in Japan and Korea. Takamiya *et al.* (1994) recognized $2n$ = 44, 65, 66, and 68 cytotypes for *I. sinensis*. Later, Takamiya *et al.* (1997) revised this earlier taxonomy recognizing the tetraploid cytotype as *I. sinensis* var. *sinensis* and the $2n$ = 65, 66, and 68 cytotypes as *I. sinensis* var. *coreana* (Y. H. Chung & H. K. Choi.) M. Takamiya, Mitsu. Wantanabe & K. Ono. In addition to chromosome number, there are differences in guard cell length, spore size, and leaf width between the tetraploid and hexaploid cytotypes. Takamiya *et al.* (1997) reported a significant difference ($p < 0.001$) in guard cell length between the tetraploid (mean = 65.2 μm) and the hexaploid (mean = 92.7 μm) cytotypes. Sporangium size in *I. sinensis* var. *sinensis* is generally smaller than in var. *coreana*. Megaspore diameter of the cytotypes ranged from 363 to 509 μm (mean = 435.1 μm) and from 375 to 538 μm (mean = 447.1 μm). The difference in leaf width can be seen in Takamiya *et al.* (1997, Table 1). Watanabe *et al.* (1996) reported that the mean microspore length of the tetraploid was 27.6 μm, whereas in the hexaploid it was 31.9 μm. After comparison of the spore and leaf morpholgy of *I. sinensis*, it appears that only the tetraploid cytotype of *I. sinensis* var. *sinensis* has been found in China.

Isoetes sinensis is extremely rare and endangered and is vulnerable due to its restricted range. Only the three small populations evaluated here are known to persist in China. Recent field studies have revealed that several previously reported populations no longer exist. This species is potentially threatened by any activities that would affect the habitat stability in the various populations. *Isoetes sinensis* can only survive if suitable habitat is preserved. At present, none of these populations occur in protected areas. Habitat modification, such as dumping sand to create beaches, pollution, and increasing urbanization in China continue to raise the risk of extinction of *I. sinensis*. More information needs to be gathered on the species' biology and habitat requirements in order to design and implement effective protection plans for saving this rare plant from extinction.

Acknowledgements

We are grateful to Wang Yong (Wuhan University, China) for preliminary editing of this manuscript. We are particularly indebted to Chen Jin-ming, Wang Jing-yuan and Liu Xing (Wuhan University, China) for their assistance with fieldwork. We also thank Guo Jing-zhe (Wuhan University, China) for assistance with image capture. This study was supported by the State Key Research and Development Plan (Grant No. 2000046805) and the National Natural Science of China(Grant No. 30370098).

Literature Cited

Chung, Y. H. and H. K. Choi. 1986. *Isoetes coreana*, a new species from Korea. Korea J. Pl. Taxon. 16:1–12.

Cox, P. A. and R. J. Hickey. 1984. Convergent megaspores evolution and *Isoetes*. Am. Nat. 124:437–441.

Foster, A. S. and E. M. Gifford. 1974. *Comparative Morphology of Vascular Plants, 2nd ed.* W H Freeman Co., San Francisco.

He, Z. C., Q. Cai, H. T. Liu, J. Q. Li and H. W. Huang. 2002. Chromosome number of *Isoetes sinensis* Palmer, a rare and endangered pteridophyta plant. J. Wuhan Bot. Res. 20:241–242.

Johanson, D. A. 1940. *Plant Microtechnique*. McGraw-Hill. New York.

Kott, L. S. and D. M. Britton. 1985. Role of Morphological Characteristics of Leaves and the Sporangial Region in the Taxonomy of *Isoetes* in Northeastern North America. Amer. Fern. J. 75:44–55.

Liu, H., Q. F. Wang and W. C. Taylor. 2005. *Isoetes orientalis* (Isoetaceae), a new hexaploid quillwort from China. Novon. 15:164–167.

Liu, X., Y. Wang and Q. F. Wang. 2002. Chromosome numbers of the Chinese *Isoetes* and their taxonomical significance. Acta Phytotax. Sin. 40:351–356.

Love, A. 1962. Cytotaxonomy of the *Isoetes echinospora* complex. Amer. Fern. J. 52:113–123.

Palmer, T. C. 1927. A Chinese *Isoetes*. Amer. Fern. J. 17:111–113.

Pang, X. A., Q. F. Wang and G. W. Robert. 2003. A Preliminary Study of Crassulacean Acid Metabolism (CAM) in the Endangered Aquatic Quillwort *Isoetes sinensis* Palmer in China. WUJNS. 8:455–458.

Pfeiffer, N. E. 1922. Monograph of the Isoetaceae. Ann. Missouri Bot. Gard. 9:79–232.

Takamiya, M., M. Watanabe and K. Ono. 1994. Biosystematic studies on the genus *Isoetes* in Japan I. Variations of the somatic chromosome numbers. J. Plant Res. 107:289–297.

Takamiya, M., M. Watanabe and K. Ono. 1997. Biosystematic Studies on the Genus *Isoetes* (Isoetaceae) in Japan. IV. Morphology and Anatomy of Sporophytes, Phytogeography, and Taxonomy. Acta Phytotax. Geobot. 48:89–122.

Takamiya, M. 2001. *Isoetes sinensis* var. *sinensis* in Korea (Isoetaceae: Pteridophyta). Fern Gaz. 16:169–177.

Taylor, W. C. and R. J. Hickey. 1992. Habitat, evolution and speciation in *Isoetes*. Ann. Missouri Bot. Gard. 79:613–622.

Tryon, R. M. and A. F. Tryon. 1982. *Ferns and Allied Plants*. Springer-Verlag, New York.

Wang, Q. F., X. Liu, W. C. Taylor and Z. R. He. 2002. *Isoetes yunguiensis* (Isoetaceae), a new basic diploid quillwort from China. Novon. 12:587–591.

Wanntorp, H. 1970. *Isoetes* in South West Africa. Svensk Bot Tidsk. 64:141–157.

Watanabe, M., M. Takamiya, T. Matsusaka and K. Ono. 1996. Biosystematic studies on the genus *Isoetes* (Isoetaceae) in Japan. III. Variability within qualitative and quantitative morphology of spores. J. Plant Res. 109:281–296.

West, C. and H. Takeda. 1915. On *Isoetes japonica* A. Br. Trans. Linn. Soc. London Bot. 8:333–376.

Woodward, F. I. 1998. Do plants really need stomata? J. Exp. Bot. 49:471–480.

Yu, Y. F. 1999. A Milestone of Wild Plant Conservation in China. Plants. 5:3–11.

American Fern Journal 96(3):75–79 (2006)

Blechnum ludificans Herter, an Overlooked Fern from South America

E. R. de la Sota

Cátedra de Morfología Vegetal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n°, 1900 La Plata, Argentina (Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina)

G. E. Giudice

Cátedra de Morfología Vegetal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n°, 1900 La Plata, Argentina

M. L. Luna

Cátedra de Morfología Vegetal, Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, Paseo del Bosque s/n°, 1900 La Plata, Argentina (Comisión de Investigaciones Científicas de la Provincia de Buenos Aires)

Abstract.—In this paper the authors reconsider the validity of the fern *Blechnum ludificans* Herter. Herbarium material was studied using stereoscopic microscopy, LM and SEM. The main diagnostic characters of *B. ludificans* are: pinnate to pinnate- pinnatisect pinnae, 25 or fewer pinnae per blade, the presence of glandular hairs on the pinnae, inframedial position of the coenosori, and entire rhizome scale margins. Illustrations of the diagnostic features of *B. ludificans* are provided.

Blechnum ludificans Herter is known only from the type specimen and grows only in República Oriental del Uruguay, South America. Its isotype provides information on its only known locality: Durazno, Blanquillo, "in fissuris rupium Gonwanicoum (arenisca roja), locis sibsiccis, soli expositis", leg. Herter IX. 1947, Herb. Hert. 99722, Pl.Ur.exs. 1985 (US 1917580). The isotype has two annotation labels: one by C.V.Morton 1964, "*Blechnum laevigatum* Cav.", and the other, "*Blechnum ludificans* Herter = *Blechnum laevigatum* Cav.?", by E.R. de la Sota 1969:. There is also an anonymous observation by hand that makes reference to its "abnormal form".

Blechnum auriculatum Cav. and *B. auriculatum* f. *mucronato-dentata* Ros. were cited by Herter (1950) as related to *B. ludificans*. According to Durán (1997) *B. auriculatum* Cav.and *B. australe* L. *f. mucronato-dentatum* Rosenst. are synonyms of *B. australe L. subsp. auriculatum* (Cav.) de la Sota.

During a field trip to Uruguay (in April 2004) the authors were unable to re-collect this fern in Durazno Department, probably due to habitat alteration by human activity. The aim of this study is to analyse the diagnostic characters of *Blechnum ludificans*, in order to evaluate the validity of the taxon proposed by Herter.

Material and Methods

The only material of *Blechnum ludificans* studied was the isotype mentioned above. In order to compare this specimen with *B. australe* subsp.

TABLE 1. Diagnostic characters of *Blechnum ludificans* and *B. australe* subsp *auriculatum*.

Characters	*B. ludificans*	*B. australe* subsp *auriculatum*
Rhizome orientation	creeping, ascending	creeping, ascending
Rhizome scales	basifixed, delotid-lanceolate, partially to totally sclerotic, entire margin	basifixed, delotid, partially to totally sclerotic, entire to laciniate margin
Blade architecture	pinnate to pinnate- pinnatisect	pinnate
Number of pinna pairs	up to 25	45 or more
Frond dimorphism	subdimorphic	subdimorphic
Blade indument	glandular hairs on rachis and pinnae surface	scarcely glandular hairs on pinnae surface
Pinna base	auriculate- mucronate	auriculate- attenuate
Indusium position	inframedial	intramarginal or medial
Spore wall sculpture	scarcely folded	scarcely folded

auriculatum, material from this species was also analysed. The pinnae were diaphanized according to the Foster technique (1934), and observations were made with a Willd M5 stereoscope and a Nikon Labophot-2 light microscope.

For LM the spores were treated with hot 3% sodium carbonate for 2 minutes and acetolyzed according to Erdtman (1960). For SEM the material was treated with hot 3% sodium carbonate (Morbelli, 1980) and coated with gold. Observations were performed with a JEOL JSMT- 100 scanning electron microscope at the Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata.

The following characters were analysed: rhizome position, scales, blade architecture, fronds dimorphism, indument, indusium position and margin, spore diameter and spore wall sculpture. Terms used to describe the indusium and rhizome position follow Lellinger (2002). Specimens examined as part of this study are listed below.

Blechnum australe L. subsp. *auriculatum* (Cav.) de la Sota

ARGENTINA: Pcia Buenos Aires, Tandil, Cerro Leones, 2/3/1946, 300 msnm, *Looser 2955* (LIL); Olavarría, Cerro "Dos Hermanas", 21/4/1947, 250 msnm, *Krapovickas 3421* (LIL); Tornquist, Sierra de la Ventana, 20/4/1945, *Krapovickas 2189* (LP); Pdo Ensenada, Punta Lara, 4/1/1940, *Dawson 933* (LIL). Pcia. Salta: Tartagal, *Meyer 916*, 12-VI-1934 (BA).

URUGUAY: Depto Durazno, Ruta 43 a 25 Km de San Gregorio de Polanco, 31/3/2004, *Giudice, Ramos Giacosa & Luna 132* (LP), Depto Tacuarembó, Gruta de los Helechos, 30/3/2004, *Giudice, Ramos Giacosa & Luna 114* (LP).

Blechnum ludificans Herter

URUGUAY: Depto. Durazno, Blanquillo, IX-1947, *Herb. Herter 99722*, (US 1917580).

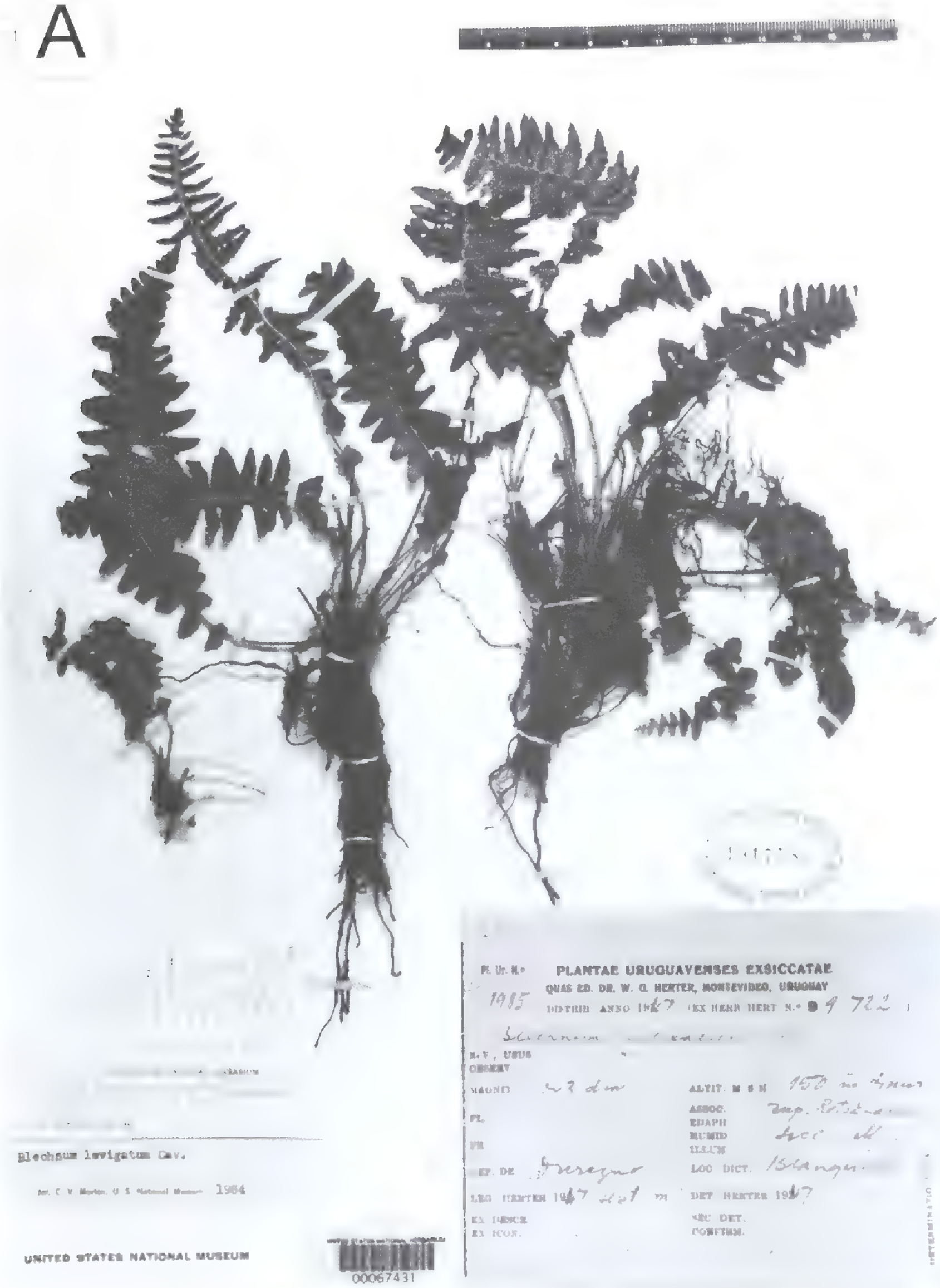


FIG. 1. Isotype *of Blechnum ludificans* (Herb. Hert. 99722, Pl.Ur.exs. 1985 [US 1917580]).

RESULTS AND DISCUSSION

The results of our morphological comparison between *Blechnum ludificans* and *B.australe subsp auriculatum* Cav., considered by Herter (1950) to be closely related, are summarized in Table 1. Although *Blechnum ludificans* is a rare and infrequent species, the characters identified in this analysis lead us to consider it a valid taxon. The spores appeared regular in shape and size and

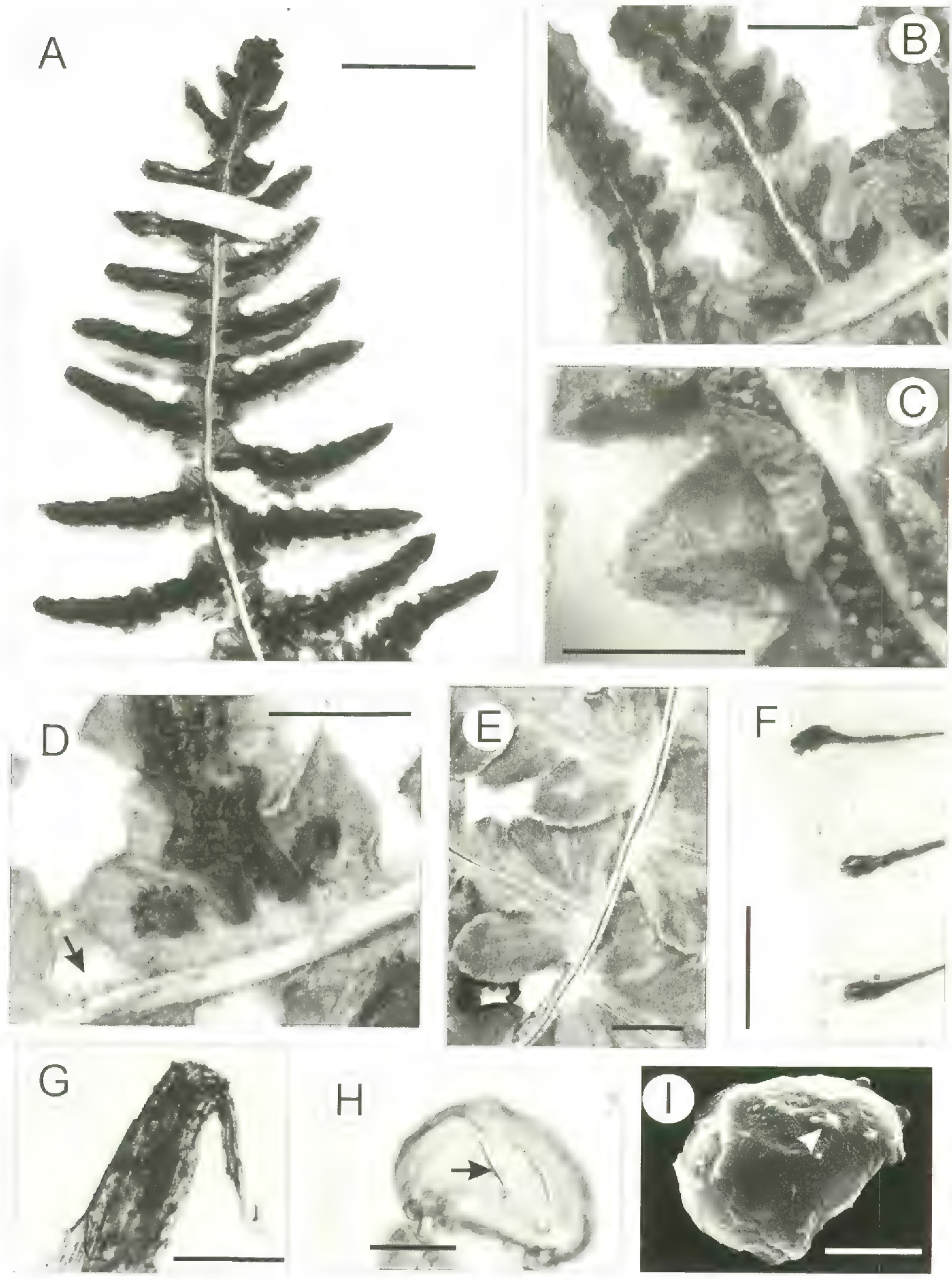


FIG. 2. *Blechnum ludificans*. **A**. Apex of fertile frond. **B**. Detail of pinnate-pinnatisect portion of fertile pinnae. **C**. Inframedial indusium position. **D**. Auriculate-mucronate segment bases; glandular hairs on the rachis indicated by arrow. **E**. Subimbricate sterile segments with auriculate-mucronate bases. **F**. Hydathodes along sterile pinna margin. **G**. Rhizome scale showing an entire margin. **H**. Spore equatorial view, occasional folds (arrow) and globules (arrowhead) are

no spore abortion was observed. According to our observations the diagnostic characters states for *Blechnum ludificans* are found in blade architecture, the number of pinnae per blade, pinnae indument, indusium position and rhizome scale margins.

The presence of pinnate to pinnate- pinnatisect fronds in *Blechnum ludificans* represent another example of blades with an additional degree of blade dissection, a character condition considered by Kramer *et al.* (1990) to be "of rare occurrence" in the genus *Blechnum*.

The following description of *Blechnum ludificans* is based on the isotype specimen at US (**Figs. 1, 2**).

Rhizome suberect, well developed and stoloniform, with scales attached by the base, deltoid-lanceolate, partially to totally sclerotic, margins entire. Fronds subdimorphic, up to 20 cm long; petioles light brown, adaxially grooved, with glandular, few-celled hairs and scattered ciliate scales with hair-like apices, attached by their bases; rachis light brown to pale yellow, adaxially grooved and with glandular hairs; blade subcoriaceous, pinnate with pinnatisect pinnae. Pinnae linear-deltoid in outline, with up to 25 pairs per blade, attenuate at the apex, with auriculate-mucronate bases and hyaline toothed margins; segments subimbricate, with lateral veins simple to once forked, ending in remarkable hydathodes, adaxial and abaxial surfaces with glandular, 2–4 celled hairs. Fertile pinnae narrower than the sterile ones; coenosori inframedial, continuous, or partially interrupted in the pinnatisect regions, indusia glabrous with erose margins. Spores monolete, polar diameter ca.19 µm, equatorial diameter ca. 31 µm; perispore folded with granular ornamentation and superficial globules of sporopolenin.

Literature Cited

Durán, M. L. 1997. Estudios morfológicos, taxonómicos y biosistemáticos en el género *Blechnum* (Blechnaceae-Pteridophyta). Ph D. dissert. (ined.), Fac. Ci. Exact. Fís. & Nat., Universidad Nacional de Córdoba, Argentina.

Foster, A. 1934. The use of tannic acid and iron chloride for staining cell walls in meristematic tissue. Stain Technol. 9:91–92.

Herter, G. 1950. Un nuevo *Blechnum* de Uruguay. Revista Sudamer. Bot. 8:162–163.

Kramer, K. U., C. Chambers and E. Hennipman. 1990. Blechnaceae. Pp. 60–68, in K. Kubitzki, ed. (K. U. Kramer and P. S. Green, vol. eds.) *The Families and Genera of Vascular Plant.* Springer-Verlag, Berlin.

Lellinger, D. B. 2002. A modern multilingual glossary for taxonomic. Pteridology 3:1–263.

Morbelli, M. A. 1980. Morfología de las esporas de las Pteridophyta presentes en la región Fuego-Patagónica, República Argentina. Opera Lilloana 28:1–138.

←
present (LM). **I.** Spore showing folded perispore with granular ornamentation and a superficial globule (arrowhead) (SEM). Bars: A: 1 cm; B, D and E: 25 mm; C: 2 mm; F: 5 mm; G: 35 µm; H and I: 10 µm

American Fern Journal 96(3):80–82 (2006)

A New Species of *Microlepia* (Dennstaedtiaceae) from Mt. Micangshan, China

Xiao-si Guo and Bin Li

Herbarium, College of Life Science, Northwest A & F University, Yangling, Shaanxi 712100, China

Abstract.—A new species, *Microlepia micangshanensis* (Dennstaedtiaceae), is described and illustrated from the northern slope of Mt. Micangshan in Hanzhong City of Shaanxi Province, China.

The genus *Microlepia* (Dennstaedtiaceae) consists of ca 70 species, distributed mainly in tropical and subtropical regions of Asia, with a few in Africa and Australia and one in the Neotropics (Wu and Ching, 1991). Ching *et al.* (1959) recorded fifty-nine species of *Microlepia* from China, mainly from the Changjiang River area and from southern and southwestern China. Recently, during the course of examination of the fern specimens deposited in WUK, a new species, *Microlepia micangshanensis*, from Mt. Micangshan was discovered.

Microlepia micangshanensis X. S. Guo *et* B. Li, ***sp. nov.*** Fig. 1.

Herba perennis, planta tota 40–60 cm alta. Rhizoma late repens, 3 mm crassum, pilis griseo-brunneis dense obtectum; frondibus distantibus. Stipes 15–25 cm longus, 3 mm diametro, brunneolus. Lamina bipinnata, lanceolata, 25–40 cm longa, ca 10 cm lata, apice attenuata; pinnis ca. 25-jugis, alternis, obliquis, petiolulatis (petiolulo ca 2 mm longo), lanceolatis, 2–7 cm longis, 1–2 cm latis, acuminatis, basi utrinque inaequaliter cuneatis, pinnatis; pinnulis (segmentis) ultimis 7–8-jugis, ovatis vel oblongis, sessilibus, basali acroscopica majore, ad 15 mm longa, 10 mm lata, pinnatifidis, obtusa, ceteris sequentibus minoribus, basiutrinque inaequalter, cuneatis, marginibus acroscopicis 3–4 lobato-incisa, marginibus basiscopicis fere integris, apice dentatis; nervis in pinnulis pinnatis; nervulis iterum pinnatis vel furcatis simplicibusve, obliquis, utrinque prominulis; textura chartacea, colore in sicco flavescente viridi, paginis utrisque subglabris, costis aut nervis persparse strigosis exceptis; rachi rachillisque supra glabris, infra dense puberulis; soris parvis ad sinum loborum positis plerumque 1–5 pro segmento, indusiis semicupliformibus, pallide brunneis, dense et longe strigosis.—TYPE. China, Shaanxi Province, Hanzhong City, Xiaonanhai, alt. 600 m, 16 Mar. 1959, *Pei-yuan Li 1060* (holotype: WUK). China, Shaanxi, Hanzhong, Xiaonanhai, alt. 650 m, 15 Mar. 1959, *Pei-yuan Li, 1044* (paratype: WUK).

Perennial herb, 40–60 cm high. Rhizome long-creeping, 3 mm wide, clothed with gray-brown hairs. Stipes 15–25 cm long, distant, 3 mm wide, brownish.

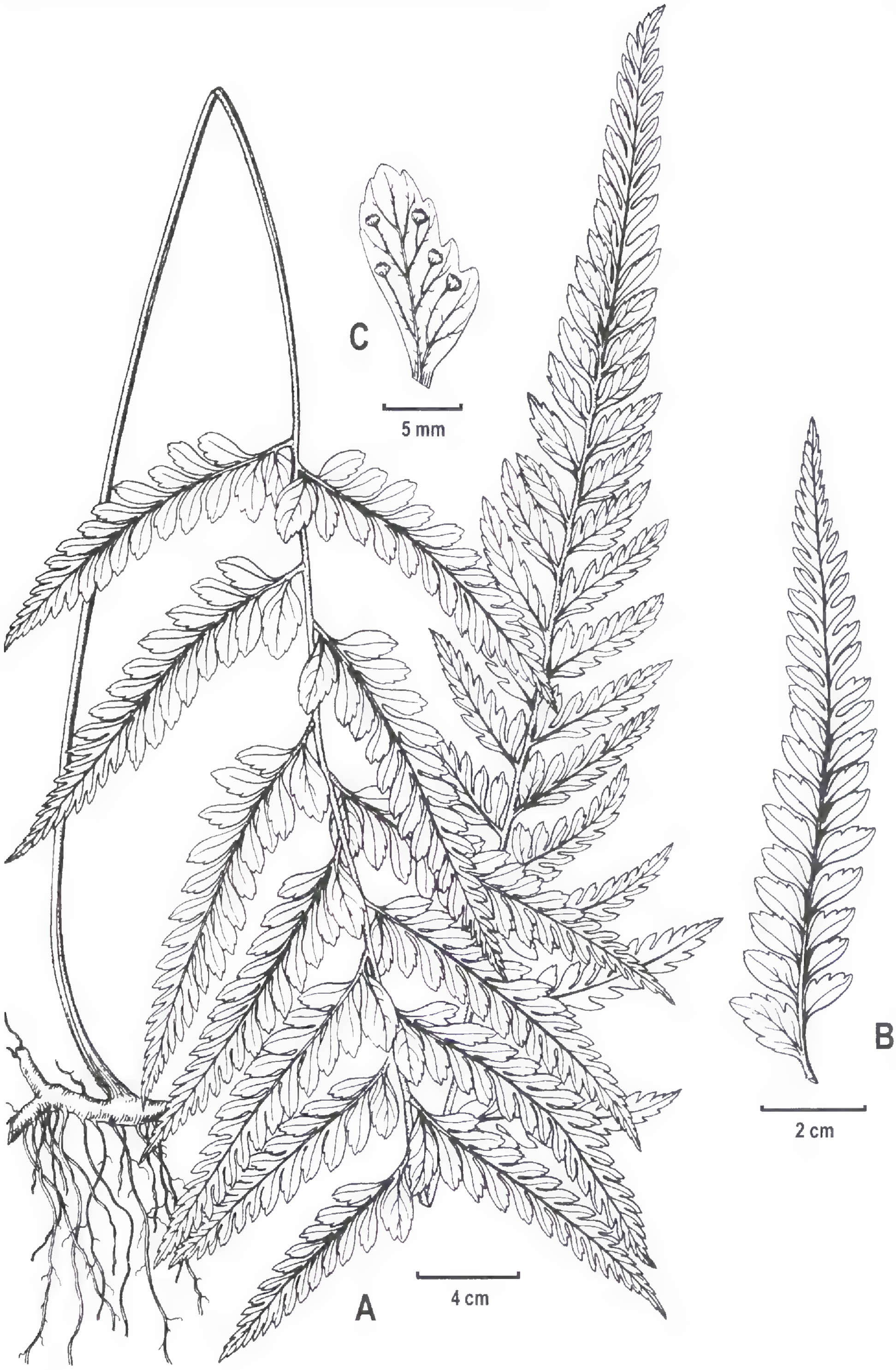


FIG. 1. Holotype of *Microlepia micangshanensis*. A. Habit. B. Pinna. C. Ultimate segment showing veins and sori.

Lamina bipinnate, lanceolate, 25–40 cm long, ca 10 cm wide, apex attenuate; pinnae ca 25 pairs, alternate, oblique, short-stalked (ca. 2 mm long), lanceolate, 2–7 cm long, 1–2 cm wide, apex acuminate, base cuneate, deeply pinnatifid; ultimate pinnules (segments) 7–8 pairs, ovate or oblong, sessile, basal acroscopic pinnule larger, usually parallel to rachis, 15 mm long, 10 mm wide, pinnatifid, apex obtuse or rounded, other pinnule smaller, about twice as long as wide, base cuneate, oblique, margins 3–4-incised on acroscopic side, nearly entire on basiscopic side, apex dentate; veins simple or forked, oblique, visible on both surfaces; fronds chartaceous when dry, dark green, strigose on vein of both surfaces; upper surface of rachis and rachillas glabrous, lower surface of rachis and rachillas densely bearing shout brown hairs; sori small, intramarginal, terminal on the veins, 1–5 on each segment; indusium thin, half cup-shaped, fixed by base and sides, brownish, strigose.

Microlepia micangshanensis grows on forest floor or on rock and is known only from Hanzhong City of Shaanxi Province, China. Hanzhong City is located in the middle of the northern slope of Mt. Micangshan.

This new species belongs to *Microlepia* based on the possession of intramarginal sori, terminal on the veins, cup-shaped indusia, attached at the base and sides, and basal acroscopic pinnules usually parallel to rachis. *Microlepia micangshanensis* is very distinct from other known species of *Microlepia* and is easily recognized by having ca 25 pairs of deeply pinnatifid pinnae. *Microlepia micangshanensis* is most closely related to *M. sino-strigosa* Ching but differs from the latter by its deeply pinnatifid pinnae, sessile pinnules, indusium shape (half cup-shaped versus reniform) and pinnule pairs (7–8 versus 12–20)

Acknowledgments

This report was supported by funds from the National Natural Sciences Foundation of China (Grant No. 30370117, 39899400, 30499340) and the special support grants from the Chinese Academy of Sciences for *Flora of China* (KSXX-SW-122). We also express our deep gratitude for critical reviews of the manuscript.

Literature Cited

Ching, R. C., S. X. Fu, C. H. Wang and G. X. Shing. 1959. Microlepia. Pp. 211–245, in S. S. Chien and W. Y. Chun, eds. *Flora Reipublicae Popularis Sinicae 3*. Science Press, Beijing.

Wu, S. H. and R. C. Ching. 1991. Microlepia. Pp. 205–206, in *Fern Families and Genera of China*. Science Press, Beijing.

American Fern Journal 96(3):83–92 (2006)

Fern Laminar Scales Protect Against Photoinhibition from Excess Light

J. E. Watkins, Jr.[1]
University of Florida, Department of Botany, Gainesville, FL 32611 USA
Akito Y. Kawahara
University of Maryland, Department of Entomology, College Park, MD 20742
Stacy A. Leicht
University of Connecticut, Department of Ecology and Evolutionary Biology, Storrs, CT 06269
Josh R. Auld
University of Pittsburgh, Department of Biological Sciences, Pittsburgh, PA 15260
Abram J. Bicksler
University of Illinois, Department of Natural Resources and Environmental Sciences, Urbana, IL 61801
Kristine Kaiser
University of Miami, Department of Biology, Coral Gables, FL 33124

Abstract.—The laminar scales of the fern *Elaphoglossum paleaceum* were studied to determine if they act as a morphological mechanism to protect leaves from excess light. We hypothesized that if scales serve this purpose, then individual plants growing in high light would have greater laminar scale density than those in low light. For our first experiment, plants from high light roadsides were collected and subjected to artificial scale removal and then exposed to super-saturating pulse of light for 30 min. We found that leaves with their scales removed exhibited significantly greater photoinhibition than leaves with scales intact. Leaves with intact scales recovered fully after twelve hours whereas those with scales removed remained photoinhibited. Scale density on the adaxial side of leaves was positively correlated with light intensity. The data from this study indicate that fern laminar scales help reduce photoinhibition and potentially function as a morphological defense against photodamage.

The function of leaf vestiture has long been a botanical curiosity. Apart from their utility for taxonomic purposes, leaf hairs and scales play many roles that directly influence plant physiology (Karabourniotis, Kyparissis, and Manetas, 1993; Lambers, Chapin III, and Pons, 1998; Karabourniotis and Bornman, 1999; Manetas, 2003; Liakopoulos, Stavrianakou, and Karabourniotis, 2006). One commonly cited mechanism is the modification of leaf boundary layers (Lambers, Chapin III, and Pons, 1998). However, leaf vestiture can also directly influence physiology related to light and can alter the internal light environment of a leaf (Karabourniotis and Bornman, 1999), and reflect visible, infra-red, or harmful UV radiation (Manetas, 2003). There may also be important tradeoffs in that while photosynthesis is reduced in pubescent leaves relative to glabrous leaves, pubescent leaves are often protected from

[1]Current address: Department of Organismic and Evolutionary Biology, Harvard University, Biological Laboratories, 16 Divinity Ave., Cambridge, MA 02138.

overheating, excessive water loss (Ehleringer and Bjorkman, 1978) and the potentially harmful effects of excessive light (Ripley, Pammenter, and Smith, 1999; Morales et al., 2002; Manetas, 2003).

Exposure to excessive light can have negative effects at multiple physiological levels. When a leaf is exposed to light, this energy can be transferred to three pathways: photosynthesis, heat, and fluorescence. While directing energy into photosynthesis is the most beneficial pathway for plants, we will focus on fluorescence in this paper. Plant physiologists use measures of fluorescence as an indicator of chlorophyll stress specifically in relation to photosystem II. Greater fluorescence results when photosystem II (PSII) reaction centers are closed or damaged and can no longer accept additional electrons, thus interrupting electron transport. Reaction centers close when they receive light in excess of that which they can transfer. Plants can dispose of this excess light energy various biochemical mechanisms. If the mechanisms in place to deal with excessive light are inadequate, photosystems can become damaged (extreme damage results in the commonly observed bleaching effect). Both excess light alone and photodamage result in Photoinhibition: the decline in photosynthesis due to exposure to excess light (Powles, 1984; Demmig *et al.*, 1987; Krause, Virgo, and Winter, 1995; Lambers, Chapin III, and Pons, 1998). If damage to the photosynthetic machinery has not occurred, photoinhibition is easily reversed following short recovery times (hours). However, if photodamage has occurred, photosynthetic recovery may take days (Demmig-Adams and Adams, 1996).

The ability of species to recover from the effects of stress associated with high light has been relatively well studied in tropical angiosperms (Mulkey and Pearcy, 1992; Lovelock, Jebb, and Osmond, 1994; Lovelock et al., 1998). In general, species recovery is closely correlated with that species' natural distribution. Lovelock *et al.* (1994) found that gap-dependent species recovered from high light stress faster than species found in dark understory habitats. A number of additional studies have demonstrated how excess light, especially when combined with other stresses, directly acts to limit species distributions (Chazdon, 1986; Groom, Baker, and Long, 1991; Kappen *et al.*, 1998; Kursar and Coley, 1999).

Given the ability of light stress to depress photosynthesis and to shape species distributions, it is not surprising that species have evolved a myriad of complex morphological and biochemical mechanisms that allow them to cope with light stress (Demmig-Adams and Adams III, 1992). These include morphological mechanisms such as leaf hairs (Karabourniotis, Bornman, and Liakoura, 1999), leaf movement or curling to avoid direct solar radiation when leaves are dry (Lebkuecher and Eickmeier, 1993; Sherwin and Farrant, 1998; Brighigna *et al.*, 2002) and protective biochemical mechanisms such as those that divert energy into pigment conversion (e.g., carotenoids and the xanthophyll cycle) (Demmig-Adams and Adams, 1996; Tausz, Hietz, and Briones, 2001a) or glycolate metabolism (Larcher, 2003)

Little is known about how ferns cope with excess light or what mechanisms they posses to do so. Biochemical mechanisms, such as xanthophyll-mediated

energy dissipation, have been tentatively demonstrated in the pteridophytes (Eickmeier, Casper, and Osmond, 1993; Tausz, Hietz, and Briones, 2001). One more commonly observed behavior in ferns is leaf curling. Leaf curling occurs in some pteridophytes as a direct result of leaf drying. Such leaf curling reduces the exposed evaporative surface which results in reduced drying rates. Yet, dehydrated leaves and excessive light can be a deadly combination. The added, and perhaps critical, benefit of leaf curling is the reduction of exposed photosynthetic tissue to incoming solar radiation. *Pleopeltis polypodiodes P. vulgare*, and *Selaginella lepidophylla* have long been known to exhibit leaf curling (Lebkuecher and Eickmeier, 1991; Muslin and Homann, 1992; Lebkuecher and Eickmeier, 1993). In the case of *S. lepidophylla*, leaf curling has been shown to specifically limit both heat and light stress (Lebkuecher and Eickmeier, 1991, 1993). The ability of *Selaginella lepidophylla* to limit stress from light and drought has been shown to play a role in this species' ability to grow in desert like habitats (Eickmeier, Casper, and Osmond, 1993). Durand and Goldstein (2001) have also shown that native tree ferns in Hawaii exhibit greater photoinhibition when subjected to high light relative to an invasive and more widespread tree fern species. Both of these studies indicate that stress may play an important role in shaping species distributions.

One potential photoprotective mechanism that has not been examined thoroughly in the ferns is the presence of laminar scales. Laminar scales may reduce excess light thereby decreasing photoinhibition and limiting damage to photosynthetic machinery. We propose two hypotheses: 1) laminar scales act as a morphological photoprotective mechanism in the fern *Elaphoglossum paleaceum*, 2) scale density in this species will increase with increasing natural light. To test these hypotheses, we examined the effects on photoinhibition of experimental scale removal coupled with exposure of de-scaled leaves to excess light. We then examined the relationship of a naturally occurring and increasing light gradient in laminar scale density.

Materials and Methods

This study was conducted at the Cuericí Biological Field Station in the San José Province, Costa Rica, *ca* 5 km northeast of the Pan American Highway (9°33′30″N, 83°39′42″W). The elevation is *ca* 2600 m, and the vegetation is upper montane wet forest (Holdridge, 1967). At this station, we studied the fern *Elaphoglossum paleaceum* (Elaphoglossaceae) (Fig. 1a) which is abundant along high-light roadsides and in the darker understory around the station. Taxonomically, the species falls within subgenus *Lepidoglossum* whose members commonly produce scales on the abaxial and adaxial surfaces of the leaf (Fig. 1b&c). These scales have no vascular connection to the sporophyte and are easily removed with no apparent damage to the sporophyte.

In the field, a 100 m transect was set up along a naturally occurring light gradient that began at a fully exposed roadside site and continued though an open area and into the forest. Three similarly aged sporophtyes were collected

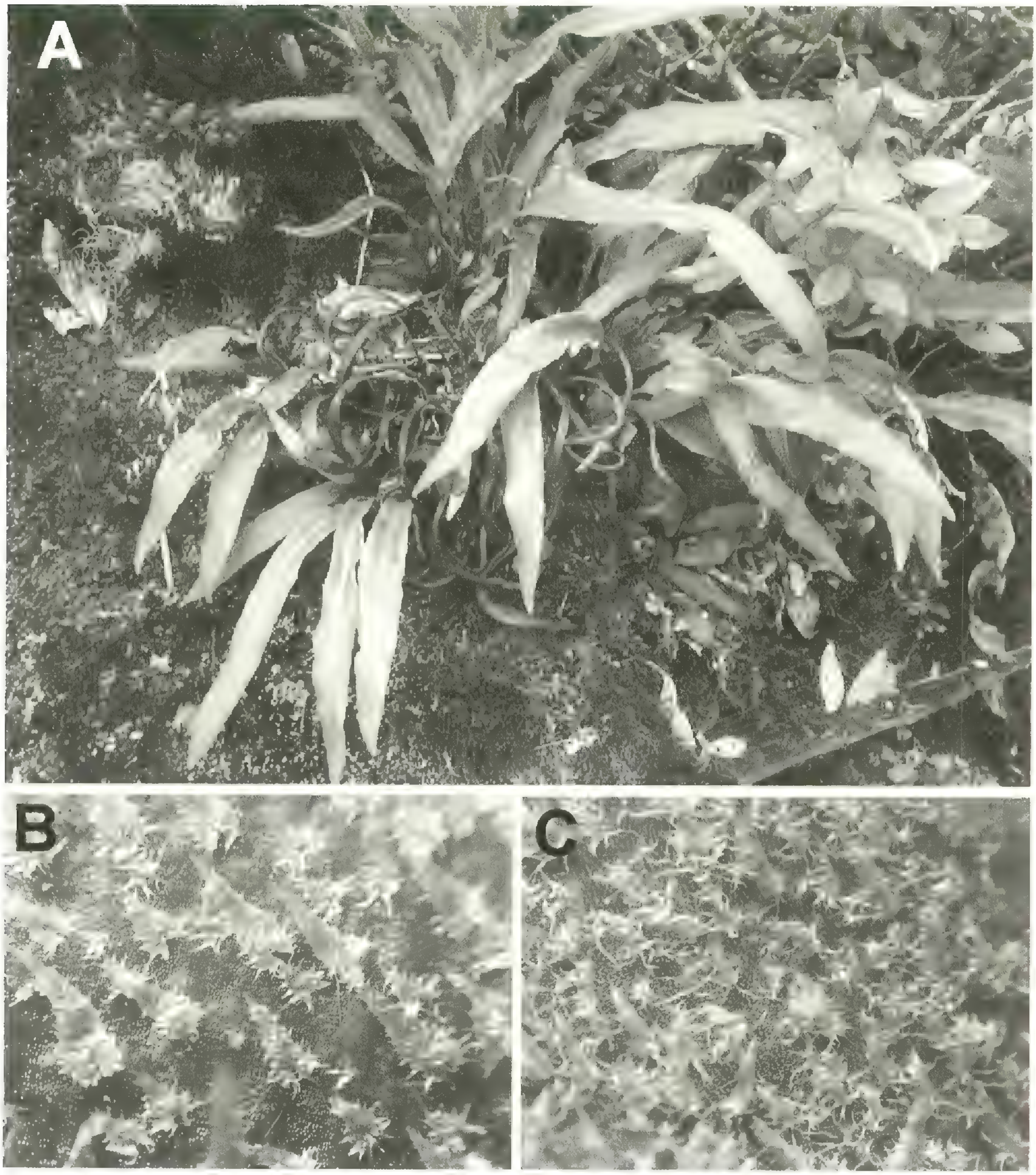


FIG. 1. *Elaphoglossum paleaceum*. A. Habit; this individual was photographed growing on a roadside in full sunlight around Cuericí Biological Field Station in Costa Rica. B. Scale morphology and density on the adaxial surface and of a typical sun leaf. C) Abaxial surface. B and C photographed at the same magnification.

from 20 of the nearest individuals at every 5 m interval along the 100 m light gradient. Scale density was quantified under a stereoscope on both the abaxial and adaxial surfaces of a 10 mm × 10 mm area from each of three sporophytes. An ANOVA was run to determine if there were differences in scale density among the leaves of individual plants. No differences were found and we therefore calculated the average abaxial and adaxial densities of each three leaf set.

To assess light environments, a digital hemispherical photograph was taken above each sampled individual with a Nikon Coolpix 950 digital camera (Melville, New York, USA) with a fisheye lens attachment. Photographs were then analyzed using the computer program Gap Light Analyzer (Frazer et al., 1999) to estimate the percentage of total transmittance. Regression analysis of the relationships between percent transmittance and scale density on both leaf surfaces was performed using the statistical software package JMP (SAS-Institute, 2005).

In order to determine whether scales act as a morphological photoprotective mechanism, ten plants were field collected from high light roadside environments with roots intact and placed in plastic bags containing 250 ml of water for two hours. Two leaves of similar ages were marked on each plant. On one frond, scales were removed using the blunt end of a spatula whereas scales on the other frond were left intact. To establish a baseline of leaf function on the marked fronds, we used an Opti-Sciences modulated flourometer (0S-500) to measure chlorophyll fluorescence. For this measurement, leaves were placed in the dark for 20 min after which we took an initial dark-adapted measurement of chlorophyll fluorescence (F_v/F_m). Plants were then exposed to a 30 min pulse of light at 2000 $\mu mol/m\text{-}^{2}/sec\text{-}^{1}$. The pulse of light was produced by MR16 Superline 50 w halogen bulbs with dichroic coated reflectors. Dichroic coated reflectors are made up of dozens of layers of thin materials that reduce the heat generated from the bulb by reflecting infrared light back into the reflector and away from the specimen.

Immediately following this exposure, chlorophyll fluorescence (F_v/F_m) was again measured in order to determine the degree of inhibition relative the pre-treatment dark-adapted measurement of F_v/F_m. To determine the degree of recovery following exposure to the light treatment, plants were placed in the dark and another dark adapted (F_v/F_m) measurement was taken 12 hr post light treatment. As a control, an additional set of plants had their scales removed but did not experience the light stress treatment. A two sample t-test was performed to determine if there were differences between the initial dark-adapted, post light stress (inhibition) treatment, and the 12 hr post treatment F_v/F_m measurements using JMP software (SAS-Institute, 2005).

Measurements of chlorophyll fluorescence have been widely used in studies to measure the general physiological condition or degree of stress that a plant may be experiencing. Standard chlorophyll fluorescence methods were followed in this study. The initial dark-adapted values of F_v/F_m mentioned above provide an estimate of the maximal quantum efficiency of Photosystem II, which in healthy, unstressed material universally converges around 0.76–0.83 (Bjorkman and Demmig, 1987). The degree of photoinhibition from the light treatment was determined by comparison to the initial dark-adapted value. Although photosynthesis is not measured directly, there is significant correlation across many studies to indicate that lowered F_v/F_m values result in lowered photosynthetic rates. Thus, measurements of F_v/F_m serve as a proxy for photosynthesis (Bjorkman and Demmig, 1987; Lambers, Chapin III, and Pons, 1998).

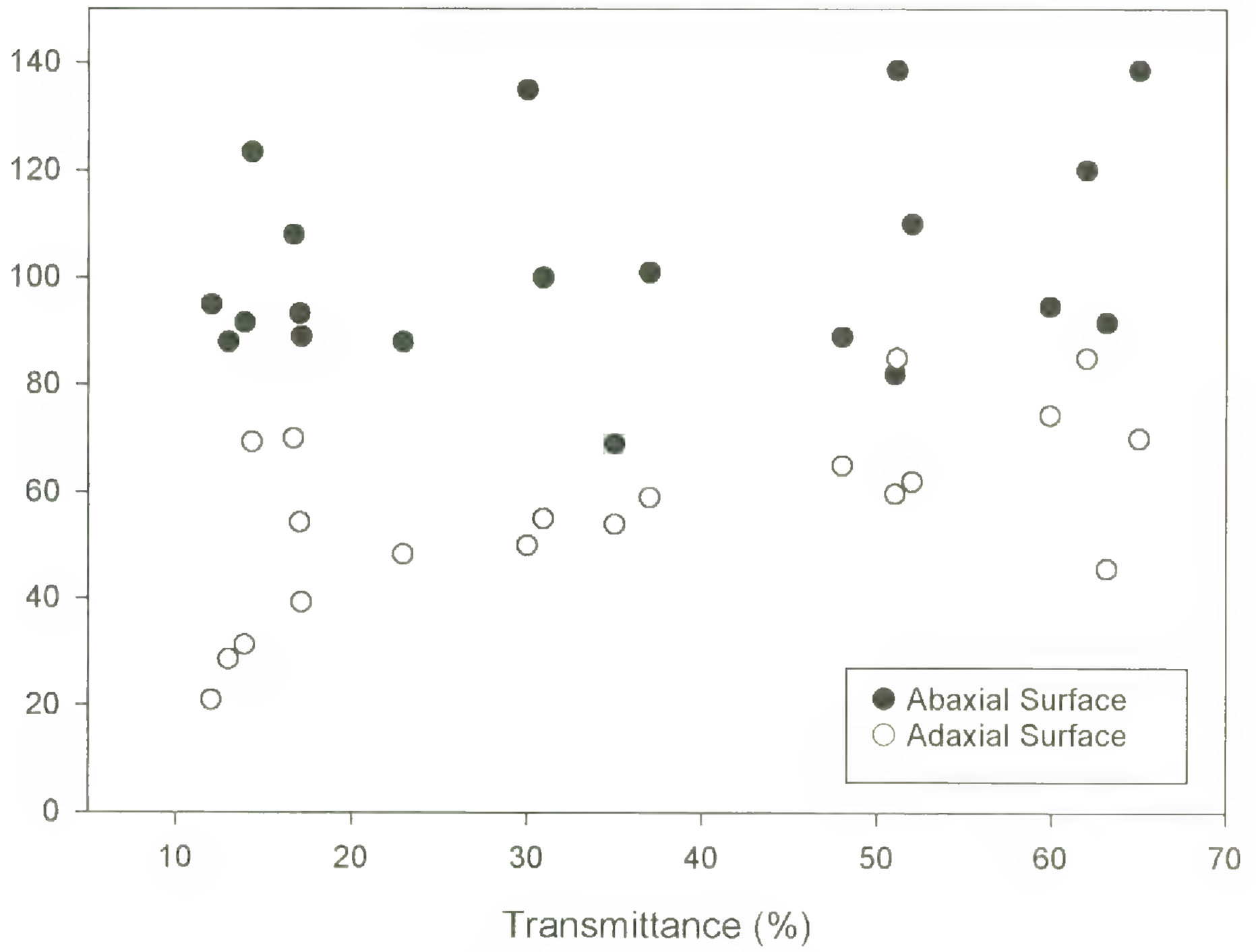


FIG. 2. The relationship between natural light transmittance and leaf scale density of *Elaphoglossum paleaceum*.

RESULTS

Regardless of light intensity of the habitat, scale density was significantly higher on the abaxial (bottom surfaces, away from exposure) than on the adaxial (top surface, facing exposure) of the leaves (data not shown, $t = 6.631$, $p < 0.0001$). As predicted, we found that adaxial scale density increased with increasing percent transmittance along our sampled light gradient (Fig. 2). The relationship was significant with a linear regression ($r^2 = 0.3843$, $p = 0.0035$), but exhibited a slightly better fit with a second order polynomial regression ($r^2 = 0.409$, $p = 0.0115$). There appeared to be no clear relationship between abaxial scale density and percent light (Fig. 2; linear regression, $r^2 = 0.0689$, $p = 0.2633$).

Initial dark adapted measurements indicated that the leaves were healthy and not experiencing stress due to transplantation into plastic bags (Fig 3a, $n = 10$, $p = 0.421$). After exposure to supersaturating light, the leaves with scales intact exhibited significantly less photoinhibition than plants with the scales removed (Fig. 3b, $n = 10$, $p < 0.0001$). Following the 12 hour post stress recovery period, leaves with scales intact exhibited significantly greater recovery than leaves with the scales removed (Fig. 3c, $n = 10$, $p < 0.0001$). There was also no significant photoinhibition in control plants with scales removed but not exposed to light stress ($n = 10$, $p = 0.524$).

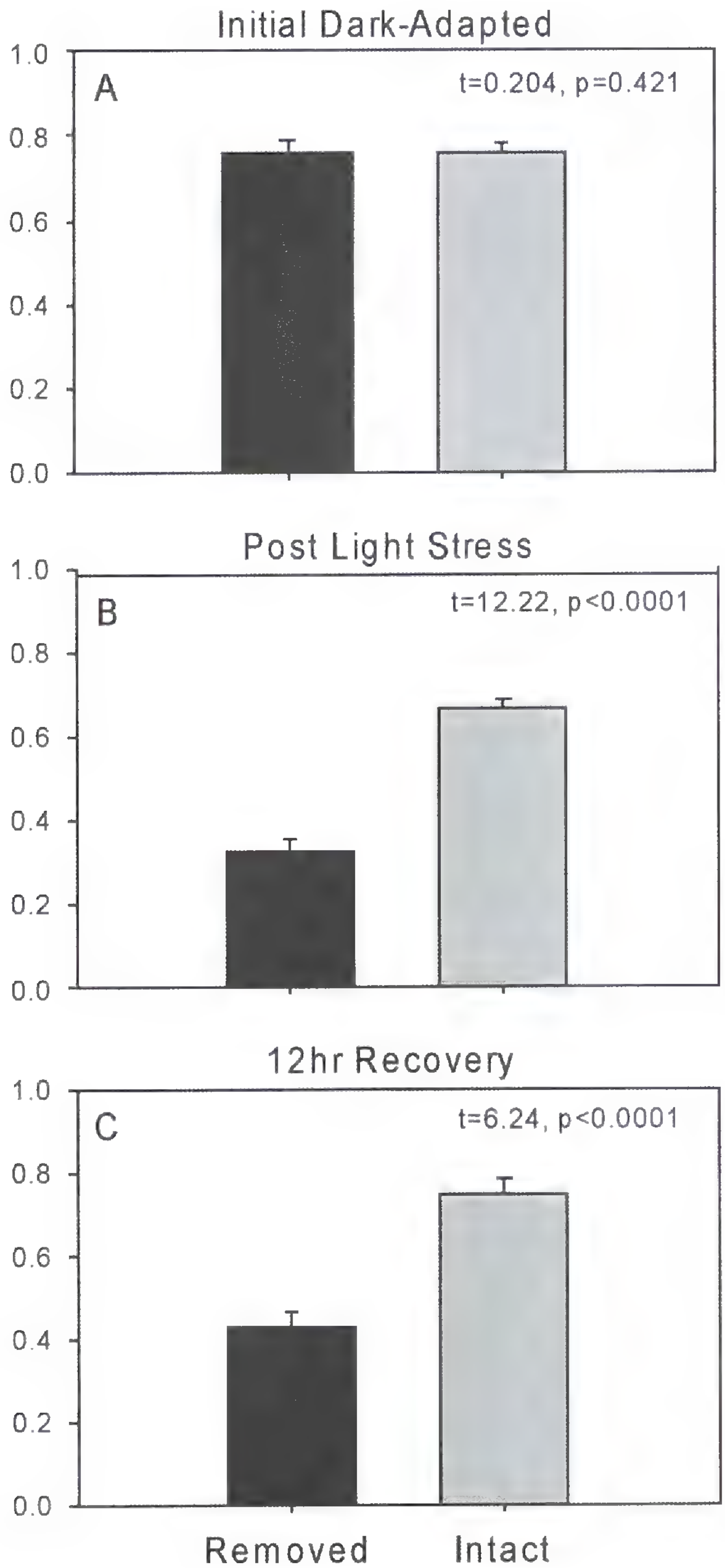


FIG. 3. Changes in photosystem II yield (F_v/F_m) from fronds of *Elaphoglossum paleaceum* with scales intact and with scales experimentally removed. A. Initial 20 min. dark adapted control values. B. F_v/F_m values after 30 min. exposure to a light level of 2000 umol/m^{-2}/sec^{-1}. C. Recovery values of F_v/F_m 12 hours post light stress. A two sample t-test (n = 10) was performed to compute differences between leaves with and without scales. Error bars are standard errors.

Discussion

The data from our study suggest a novel role for fern laminar scales: that of photoprotection. Plants where the scales were removed had significantly lower F_v/F_m values after high light stress, which is indicative of greater photoinhibition, compared to leaves with scales intact. Additionally, leaves with scales intact exhibited slightly, but not significantly lower F_v/F_m values after high light stress in comparison to the dark-adapted values (Fig. 3a intact compared to Fig. 3b intact values; analysis not shown). The significantly reduced rate of F_v/F_m recovery in leaves where scales have been removed (Fig. 1c) indicates that these plants likely experienced photodamage. Taken together, these data indicate that the presence of fern laminar scales in *Elaphoglossum paleaceum* clearly protects plants from photoinhibition and photodamage that occur when plants are exposed to excess light.

This hypothesis is further supported by the positive relationship of scale density with natural light intensity. Adaxial scale density was positively correlated with an individual plant's natural light regime where plants in high light had greater scale density that plants in low light. This follows our prediction that if laminar scales act as a morphological photoprotective mechanism then plants in high light habitats should exhibit greater investment in such structures.

Elaphoglossum paleaceum is additionally intriguing in that it exhibits leaf curling during times of extreme drought (pers. obs.). The differential pattern of scale density between the abaxial and adaxial surfaces combined with leaf curling may be explained as a last line of morphological defense in times of drought. During leaf curling, the abaxial surface curls upwards forming a tube that completely hides the adaxial surface (pers. obs.). This mechanism presents the more densely covered abaxial surface to full exposure incoming solar radiation. This curling can reduce surface exposure and slow evapotranspiration and thus potentially reduce or eliminate the damaging effects of extreme light. In times of high light without drought or with limited drought, adaxial scale density may be sufficient to protect the plant from extreme photoinhibition and photodamage. The interaction of scale density with light intensity suggests that variation in this character may be driven by light and provides some adaptive value.

There are numerous other species of *Elaphoglossum* in the flora of this region that lack scales and leaf curling, yet grow alongside *Elaphoglossum paleaceum*. It is likely that different strategies have evolved among these species with the glabrous taxa relying on biochemical mechanisms. Future studies would benefit greatly from inclusion of these species and from investigation of protective pigments.

Acknowledgments

This study was done during the Tropical Ecology course taught under the auspices of the Organization for Tropical Studies (OTS 04–01). The course was supported by a grant to OTS from the Andrew W. Mellon Foundation. We thank Robbin Moran, Chad Husby, and Grace Crummer for comments on an earlier version of the manuscript.

Literature Cited

Bjorkman, O. and B. Demmig. 1987. Photon yield of O-2 evolution and chlorophyll fluorescence characteristics at 77-K among vascular plants of diverse origins. Planta 170:489–504.

Brighigna, L., A. Bennici, C. Tani and G. Tani. 2002. Structural and ultrastructural characterization of *Selaginella lepidophylla*, a desiccation-tolerant plant, during the rehydration process. Flora 197:81–91.

Chazdon, R. L. 1986. Light variation and carbon gain in rain forest understory palms. J. Ecol. 74:995–1012.

Demmig-Adams, B. and W. W. Adams III. 1992. Photoprotection and other responses of plants to high light stress. Ann. Rev. Plant Physiol. Plant Mol. Biol. 43:599–626.

Demmig-Adams, B. and W. W. Adams. 1996. The role of xanthophyll cycle carotenoids in the protection of photosynthesis. Trends in Plant Sci. 1:21–26.

Demmig, B., K. Winter, A. Kruger and F. C. Czygan. 1987. Photoinhibition and zeaxanthin formation in intact leaves - a possible role of the xanthophyll cycle in the dissipation of excess light energy. Plant Physiol. 84:218–224.

Durand, L. Z. and G. Goldstein. 2001. Photosynthesis, photoinhibition, and nitrogen use efficiency in native and invasive tree ferns in Hawaii. Oecologia 126:345–354.

Ehleringer, J. R. and O. Bjorkman. 1978. Pubescence and leaf spectral characteristics in a desert shrub, *Encelia farinosa*. Oecologia 36:151–162.

Eickmeier, W. G., C. Casper and C. B. Osmond. 1993. Chlorophyll fluorescence in the resurrection plant *Selaginella lepidophylla* (Hook & Grev) Spring during high-light and desiccation stress, and evidence for zeaxanthin-associated photoprotection. Planta 189:30–38.

Frazer, G. W., C. D. Canham, P. Sallaway and D. Marinakis. 1999. *Gap Light Analyzer*. Simon Fraser University & Institute for Ecosystem Studies, Burnaby, British Columbia, Canada & Millbrook, New York.

Groom, Q. J., N. R. Baker and S. P. Long. 1991. Photoinhibition of Holly (*Ilex aquifolium*) in the Field During the Winter. Physiol. Plantarum 83:585–590.

Holdridge, L. R. 1967. *Life Zone Ecology*. Tropical Science Center, San Jose, Costa Rica.

Kappen, L., B. Schroeter, T. G. A. Green and R. D. Seppelt. 1998. Chlorophyll a fluorescence and CO2 exchange of *Umbilicaria aprina* under extreme light stress in the cold. Oecologia 113:325–331.

Karabourniotis, G. and J. F. Bornman. 1999. Penetration of UV-A, UV-B and blue light through the leaf trichome layers of two xeromorphic plants, olive and oak, measured by optical fibre microprobes. Physiol. Plantarum 105:655–661.

Karabourniotis, G., A. Kyparissis and Y. Manetas. 1993. Leaf hairs of *Olea europaea* protect underlying tissues against ultraviolet-B radiation damage. Environ. Ex. Bot. 33:341–345.

Karabourniotis, G., J. F. Bornman and V. Liakoura. 1999. Different leaf surface characteristics of three grape cultivars affect leaf optical properties as measured with fibre optics: possible implication in stress tolerance. Aus. J. Plant Physiol. 26:47–53.

Krause, G. H., A. Virgo and K. Winter. 1995. High susceptibility to photoinhibition of young leaves of tropical forest trees. Planta 197:583–591.

Kursar, T. A. and P. D. Coley. 1999. Contrasting modes of light acclimation in two species of the rainforest understory. Oecologia 121:489–498.

Lambers, H., F. S. Chapin III and T. L. Pons. 1998. *Plant Physiological Ecology*. Springer-Verlag, New York.

Larcher, W. 2003. *Physiological Plant Ecology, Ecophysiology and Stress Physiology of Functional Groups, 4th edition*. Springer-Verlag, Berlin.

Lebkuecher, J. G. and W. G. Eickmeier. 1991. Reduced photoinhibition with stem curling in the resurrection plant *Selaginella-Lepidophylla*. Oecologia 88:597–604.

Lebkuecher, J. G. and W. G. Eickmeier. 1993. Physiological benefits of stem curling for resurrection plants in the field. Ecology 74:1073–1080.

Liakopoulos, G., S. Stavrianakou and G. Karabourniotis. 2006. Trichome layers versus dehaired lamina of *Olea europaea* leaves: differences in flavonoid distribution, UV-absorbing capacity, and wax yield. *Environ. Ex. Bot.* 55:294–304.

Lovelock, C. E., M. Jebb and C. B. Osmond. 1994. Photoinhibition and recovery in tropical plant species - response to disturbance. Oecologia 97:297–307.

Lovelock, C. E., T. A. Kursar, J. B. Skillman and K. Winter. 1998. Photoinhibition in tropical forest understory species with short- and long-lived leaves. Funct. Ecol. 12:553–560.

Manetas, Y. 2003. The importance of being hairy: the adverse effects of hair removal on stem photosynthesis of *Verbascum speciosum* are due to solar UV-B radiation. New Phytologist 158:503–508.

Morales, F., A. Abadia, J. Abadia, G. Montserrat and E. Gil-Pelegrin. 2002. Trichomes and photosynthetic pigment composition changes: responses of *Quercus ilex* subsp. *ballota* (Desf.) Samp. and *Quercus coccifera* L. to Mediterranean stress conditions. Trees-Struct. Funct. 16:504–510.

Mulkey, S. S. and R. W. Pearcy. 1992. Interactions between acclimation and photoinhibition of photosynthesis of a tropical forest understory herb, *Alocasia macrorrhiza*, during simulated canopy gap formation. Funct. Ecol. 6:719–729.

Muslin, E. and P. Homann. 1992. Light as a hazard for the desiccation-resistant "resurrection" fern *Polypodium polypoides* L. Plant Cell and Environ, 81–89.

Powles, S. B. 1984. Photoinhibition of Photosynthesis Induced By Visible-Light. Annu. Rev. Plant Physiol. Plant Mol. Biol. 35:15–44.

Ripley, B. S., N. W. Pammenter and V. R. Smith. 1999. Function of leaf hairs revisited: The hair layer on leaves *Arctotheca populifolia* reduces photoinhibition, but leads to higher leaf temperatures caused by lower transpiration rates. J. Plant Physiol. 155:78–85.

SAS-Institute. 2005. *JMP User's Guide*. SAS Institute, Inc., Cary.

Sherwin, H. W. and J. M. Farrant. 1998. Protection mechanisms against excess light in the resurrection plants *Craterostigma wilmsii* and *Xerophyta viscosa*. J. Plant Growth Regul, 203–210.

Tausz, M., P. Hietz and O. Briones. 2001. The significance of carotenoids and tocopherols in photoprotection of seven epiphytic fern species of a Mexican cloud forest. Aus. J. Plant Physiol. 28:775–783.

American Fern Journal 96(3):93–99 (2006)

Shorter Notes

Kaempferol 3-*O*-(acetylrutinoside), a New Flavonoid and Two New Fern Constituents, Quercetin 3-*O*-(acetylglucoside) and 3-*O*-(acetylrutinoside) from *Dryopteris villarii*.—The most widespread analysis of flavonoids of the genus *Dryopteris* was carried out by Hiraoka (Biochem. Syst. Ecol. 6:171–175. 1978) who studied eighteen Japanese *Dryopteris* species belonging to sections *Dryopteris* and *Erythrovariae*; this work led to the identification of eight flavonol glycosides based on kaempferol (3-*O*-glucoside, 7-*O*-arabinoside, 7-*O*-rhamnoside, 3-*O*-rhamnoglucoside, 3-*O*-rhamnodiglucoside, 3-*O*-xylorhamnoside, 3-*O*-rhamnoglactoside-7-*O*-rhamnoside and 3-*O*-rhamnoarabinoside-7-*O*-arabinoside) together with luteolin 7-*O*-glucoside, quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoglucoside, two flavanone *O*-glucosides (naringenin 7-*O*-glucoside and eriodictyol 7-*O*-arabinoside) and three *C*-glucosylflavones (vitexin, orientin and vitexin 7-*O*-glucoside). On the basis of flavonoid distribution, Hiraoka (1978) suggested that sections *Dryopteris* and *Erithrovariae* may be divided into three and two groups respectively; these divisions agreed with those based on morphological and cytological data. In addition, the flavonoid data of Hiraoka showed that *Dryopteris watanabei* Kurata (which has been revealed to be a natural hybrid between *D. crassirhizoma* Nakai and *D. uniformis* Makino by cytological analysis) has all the flavonoids of both parental species; this result resembles the additive inheritance of chemical characters showed by Smith and Levin in the original paper (Amer. J. Bot. 50:952–958. 1963) on Appalachian *Asplenium* hybrids. 3-desoxyanthocyanins (mainly as apigenidin and luteolinidin 5-*O*-glucosides) were found in red sori of *Dryopteris erythrosora* (D.C. Eaton) O. Kuntze by Harborne (Phytochemistry 5:589–600. 1966). As shown in a review by Markham (pp. 427–468, *in* J.B. Harborne, ed. *The Flavonoids, Advances in Research* since 1980, Chapman and Hall, London and New York. 1988) a kaempferol 3-*O*-glycoside and a quercetin 3-*O*-glycoside were found in the gamethophyte and the sporophyte of *Dryopteris intermedia* (Muhl. *ex* Willd.) Gray and *D. marginalis* (L.) Gray, whereas an unusual flavonoid (3,7,3′,4′-tetrahydroxy-5-acetoxyflavan in which the methyl group of acetoxyl is further bound to C-4 of C-ring) was isolated from *Dryopteris filix-mas* (L.) Schott and kaempferol 7-*O*-(6′′-succinylglucoside) was found in four *Dryopteris* species. No study appears to have been made of flavonoids of *Dryopteris villarii* (Bellardi) Schinz & Thell.

Ten flavonoids (I–X) have been isolated from aerial parts of *Dryopteris villarii* collected in the Botanic Garden of the University of Naples (Italy). The fern was identified by Dr. R. Nazzaro (University of Naples). The flavonoids were isolated from an ethanolic extract of aerial parts of *Dryopteris villarii* by preparative paper chromatography in BAW (*n*-butanol-acetic acid-water, 4:1:5, upper phase), 15% HOAc (acetic acid) and BEW (*n*-butanol-ethanol-water,

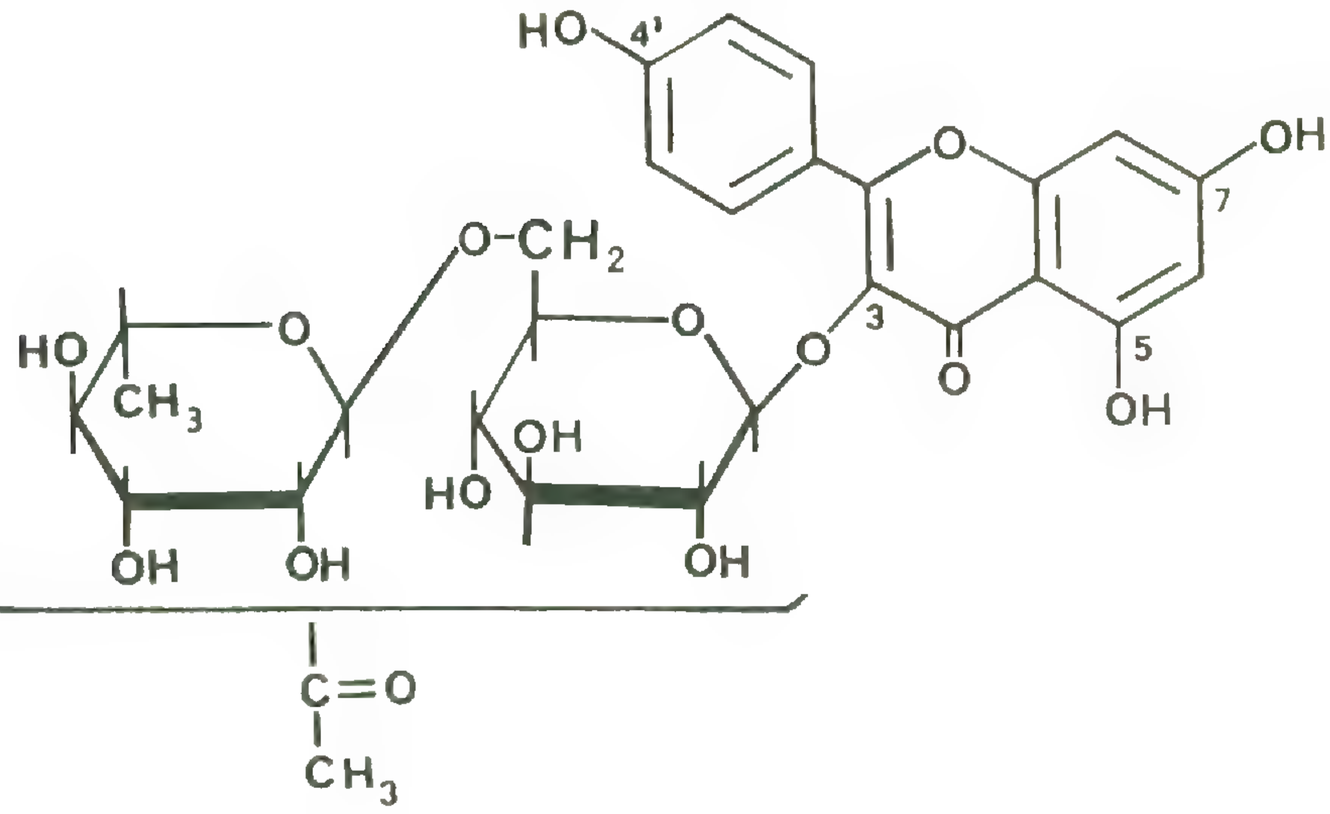


FIG. 1. Kaempferol 3-*O*-(acetylrutinoside).

4:1:2.2). Further purification was carried out by Sephadex LH-20 column chromatography eluting with methanol.

Color reactions (brown to yellow in UV+NH_3), R_f values (0.59 in BAW; 0.56 in 15% HOAc) and ultraviolet spectral analysis in the presence of the customary shift reagents (λ_{max} (nm) (MeOH) 265, 299 (sh), 351; +$AlCl_3$ 273, 299 (sh), 346, 395; +$AlCl_3$/HCl 272, 298 (sh), 346, 394; +NaOAc 273, 304, 376; +NaOMe 274, 325, 399) suggested that flavonoid (I) may be a flavonol glycoside with free hydroxyl groups at positions 5, 7 and 4′. Total acid hydrolysis (2N HCl; 1 hr at 100° C) gave kaempferol, D-glucose and L-rhamnose; controlled acid hydrolysis (10% HOAc; 3.5 hr under reflux) gave kaempferol, D-glucose, L-rhamose and the disaccharide rutinose (*O*-α-L-rhamnosyl-(1→6)-D-glucose). Electrospray mass spectrum showed a pseudomolecular ion at m/z 659 $[M+Na]^+$ and an ion at m/z 287 (aglycone); these data show that flavonoid (I) is kaempferol 3-*O*-(acetylrutinoside) (Fig. 1), a new natural product; this is the first report of an acylated flavonoid glycoside from ferns belonging to the genus *Dryopteris*.

Flavonoid (II) was identified as quercetin 3-*O*-(acetylglucoside) by ultraviolet spectral analysis in the presence of the customary shift reagents, total acid hydrolysis (which gave quercetin and D-glucose) and electrospray mass spectrum which showed a pseudomolecular ion at m/z 553 $[(M+H)+2Na]^+$ and an ion at m/z 302 (aglycone). Quercetin 3-*O*-(acetylglucoside) is a new constituent of ferns. This flavonoid has been found in *Plumeria floribunda* Müll.Arg. and in *Helenium hoopesii* A. Gray by Wagner *et al.* (Phytochemistry 10:2547–2548. 1971); subsequently quercetin 3-*O*-(6′′-acetylglucoside) has been isolated from needles of *Pinus* spp. (Pinaceae) as shown in a review by Harborne (pp. 261–311, *in* J.B. Harborne and T.J. Mabry, eds. *The Flavonoids: Advances in Research*, Chapman and Hall, London and New York. 1982). Each of these two compounds may or may not be the same as flavonoid (II) from *Dryopteris villarii*.

Flavonoid (III) was identified as quercetin 3-*O*-(acetylrutinoside) by ultraviolet spectral analysis in the presence of the customary shift reagents,

total acid hydrolysis (which gave quercetin, D-glucose and L-rhamnose), controlled acid hydrolysis (which gave quercetin, D-glucose, L-rhamnose and rutinose) and electrospray mass spectrum which showed a pseudomolecular ion at m/z 675 $[M+Na]^+$ and an ion at m/z 303 (aglycone). Quercetin 3-*O*-(acetylrutinoside) is a new fern constituent; this flavonoid has previously been found only in *Patersonia* species (Iridaceae) by Williams *et al.* (Phytochemistry 28:1891–1896, 1989). Acetylated flavonoid glycosides have not been reported previously from the genus *Dryopteris*.

As pointed out in a review by Harborne (pp. 337–385, *in* J.B. Harborne, ed. *The Flavonoid, Advances in Research since 1986*, Chapman and Hall, London. 1994) acetyl groups may be lost by acid hydrolysis since acetic acid is volatile; the increasing number of acetylated flavonoid glycosides reported from plants in recent years may be due to the use of mass spectrometry and NMR spectroscopy.

Flavonoids (IV–VII) have been identified as kaempferol 3-*O*-rutinoside (nicotiflorin) (IV), quercetin 3-*O*-rutinoside (rutin) (V), kaempferol 3-*O*-glucoside (astragalin) (VI) and quercetin 3-*O*-glucoside (isoquercitrin) (VII) by the above methods. Kaempferol 3-*O*-rutinoside is here reported for the first time from the genus *Dryopteris* although kaempferol 3-*O*-rhamnosylglucoside (interglycosidic link not determined) has been found in ten fern species belonging to this genus by Hiraoka (1978). In addition, kaempferol 3-*O*-rutinoside has previously been identified in nine species of *Adiantum*, two species of *Hemionitis*, *Loxsoma cunninghamii* R. Br., *Loxsomopsis costaricensis* Christ, *Paesia anfractuosa* (Christ) C. Chr., *Thelypteris palustris* Schott and in *Diplazium nipponicum* Tagawa as shown in a review by Markham (1988) and in a review by Imperato (pp. 39–75, in *Current Topics in Phytochemistry*, Research Trends, Trivandrum. 2000); recently this flavonoid has been found in *Pteris vittata* L. by Imperato (Amer. Fern J. 94:159–162. 2004). Rutin (V) is here reported for the first time from the genus *Dryopteris* but quercetin 3-*O*-rhamnoglucoside (interglycosidic link not determined) has been found in *Dryopteris erythrosora*, *D. championii* (Benth.) Ching and *D. gymnophylla* (Baker) C. Chr. by Hiraoka (1978). As shown in a review by Markham (1988) rutin has previously been identified in ten species of *Adiantum*, five species of *Gymnopteris*, all four species of *Bommeria*, two species of *Hemionitis*, the genus *Trachypteris*, *Paesia anfractuosa*, *Pteridium aquilinum* L. Kuhn and *Loxsoma cunninghamii*; more recently rutin has been found in *Polypodium decumanum* Willd. by Vasange *et al.* (Planta Med. 63:511–517. 1997) and in *Pteris vittata* by Imperato and Telesca (Amer. Fern J. 90:42–47. 2000).

In the genus *Dryopteris*, kaempferol 3-*O*-glucoside (VI) and quercetin 3-*O*-glucoside (VII) have been found in seventeen species and sixteen species respectively by Hiraoka (1978); these two flavonoids have been identified in a large number of fern species as shown in a review by Markham (1988) and in a review by Imperato (2000).

Flavonoids (VIII-X) were identified as apigenin (VIII), kaempferol (IX) and quercetin (X) by polyamide TLC and paper chromatography. Kaempferol and

quercetin are here reported for the first time from the genus *Dryopteris* whereas apigenin has previously been found in a single species of this genus, *D. setigera* by Voirin (Ph. D. thesis, University of Lyon. 1970). As shown in a review by Markham (1988) and in a review by Imperato (2000) apigenin has previously been found in a number of fern species belonging to *Pteridium, Notholaena, Hymenophyllum, Pteris and Cheilanthes* whereas kaempferol together with quercetin have been found in a number of fern species belonging to *Asplenium, Blechnum, Hymenophyllum, Marattia, Anemia, Lygodium and Mohria*; in addition kaempferol has been found in *Notholaena* and *Trichomanes* whereas quercetin has been identified in the genus *Botrychium*.

It is of interest to note that apigenin, kaempferol and quercetin in *Dryopteris villarii* are not "external" flavonoids (i.e. flavonoid aglycones occurring on outside of the fronds) because Wollenweber *et al.* (Phytochemistry 48: 931–939. 1998) reported the absence of flavonoids among the compounds produced by external glands of leaves of *D. villarii* and *D. arguta* (Kaulf.) Maxon; Wollenweber *et al.* (1998) found that the external glands of these two ferns produce acylphloroglucinols whereas Widen *et al.* (Botanica Helvetica 101:77–120. 1991) identified acylphloroglucinols in rhizomes of *D. villarii* and *D. arguta*; the acylphloroglucinol composition of external glands is different from that of rhizomes. Wollenweber *et al.* (1998) suggested that acylphloroglucinols may have an ecophysiological function and it has been reported by Clarke and Harvey (pp. 197–198, *Veterinary Toxicology, 2nd ed.*, Ballaire Tindall, London. 1981) that rhizome acylphloroglucinols of *Dryopteris filix-mas* produce neurotoxic effects in cattle eating rhizomes and fronds of this fern.

The author thanks Università della Basilicata for financial support. Mass spectral data were provided by CNR/ISA (Avellino, Italy). FILIPPO IMPERATO, Dipartimento di Chimica, Università della Basilicata, 85100 Potenza, Italy.

***Deparia longipes* (Woodsiaceae) Native to Taiwan.**—*Athyriopsis longipes* Ching is characterized by its peculiar long-creeping rhizome, herbaceous leaf texture, and serrate pinnule margin (Ching, Acta Phytotax. Sin. 41–85. 1964). It has been recorded only from Sichuan, Yunnan and Hunan provinces in China (Ching, 1964; Chu, Fl. Republ. Pop. Sin. 3(2): 1–566. 1999); however, a large wild population of it has been discovered at a site in Taichung, Suyuanyakou in Taiwan (Fig 1, 2). This is the first record of this species outside mainland China. The site is located (Fig. 2) approximately 800 km east of the nearest known locality at Ling Xian, Hunan province in China (Chu, 1999). We found many individuals growing on forest margins along trails or roadsides. Two plants were collected for cytological analysis. The vouchers (020606007 and 020606008) were deposited in the Herbarium of the Department of Botany, Graduate School of Science, Kyoto University (KYO).

Cytological observation of *Athyriopsis longipes* showed a chromosome number of $2n = 80$ (Fig. 3) at its newly discovered location. This number is

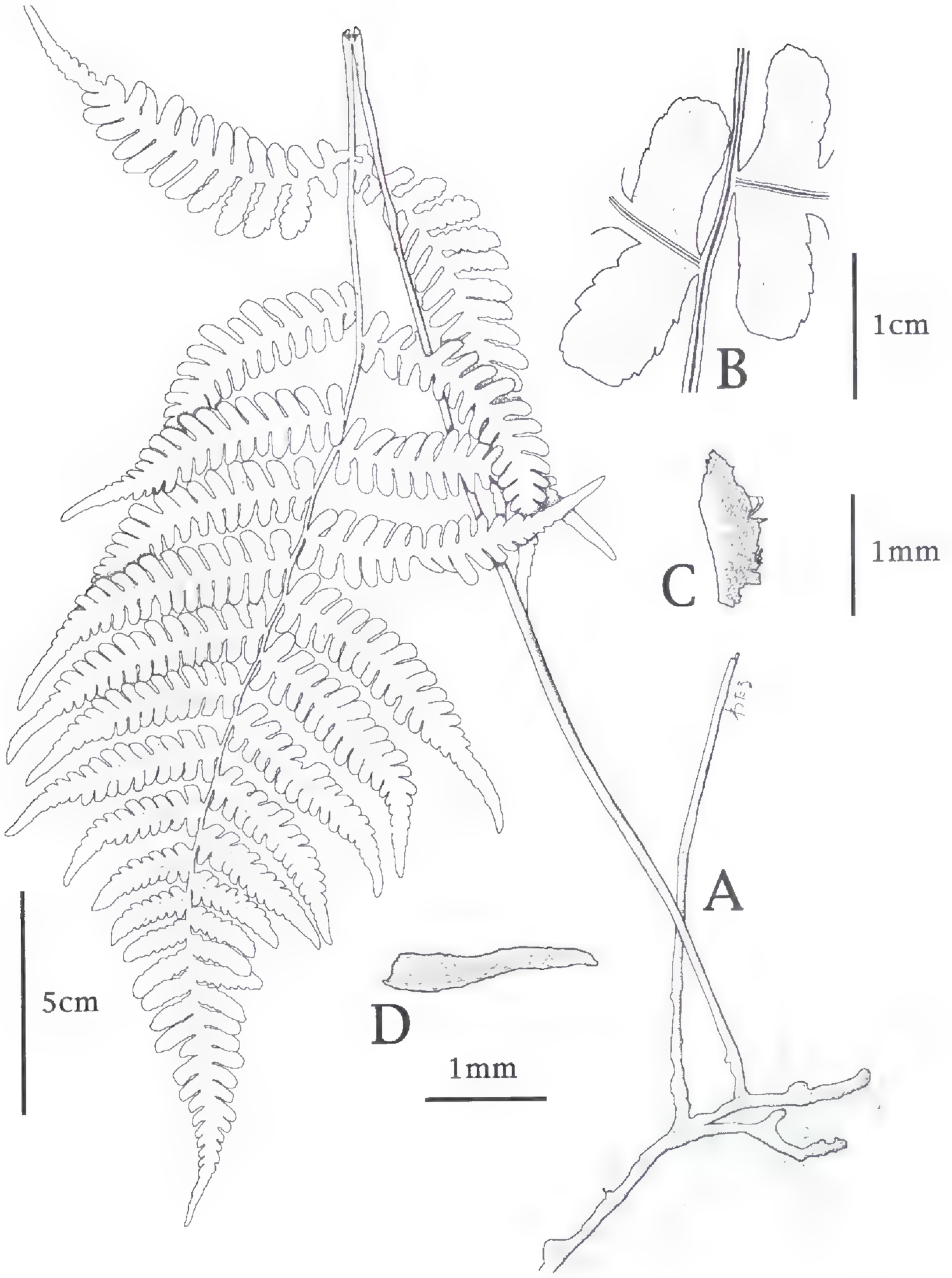


FIG. 1. *Deparia longipes*. A) Frond. B) Central lamina showing the shallow rachis groove which does not open at the junctions with the costae. C) indusium. D) Lamina scale.

regarded as diploid (Takamiya, *Index to Chromosomes of Japanese Pteridophyta*, Tokyo, Pterid. Soc., 1996).

Hooker and Greville (Icones Filicum 2: t. 154) established *Deparia* in 1829 based on *Deparia prolifera*. Kato (Bot. Mag. Tokyo 90: 23–40. 1977; J. Fac. Sci. Univ. Tokyo, Sect. 3, 13: 375–430. 1984) emphasized the similarities of the articulate hairs, leaf architecture, and leaf segmentation of *Deparia*, *Athyriopsis*, *Dryoathyrium*, and *Lunathyrium*. He considered that the monotypic genus *Deparia* is congeneric with the other three genera, and relegated *Athyriopsis sensu* Ching (1964) to one of the four sections of the genus

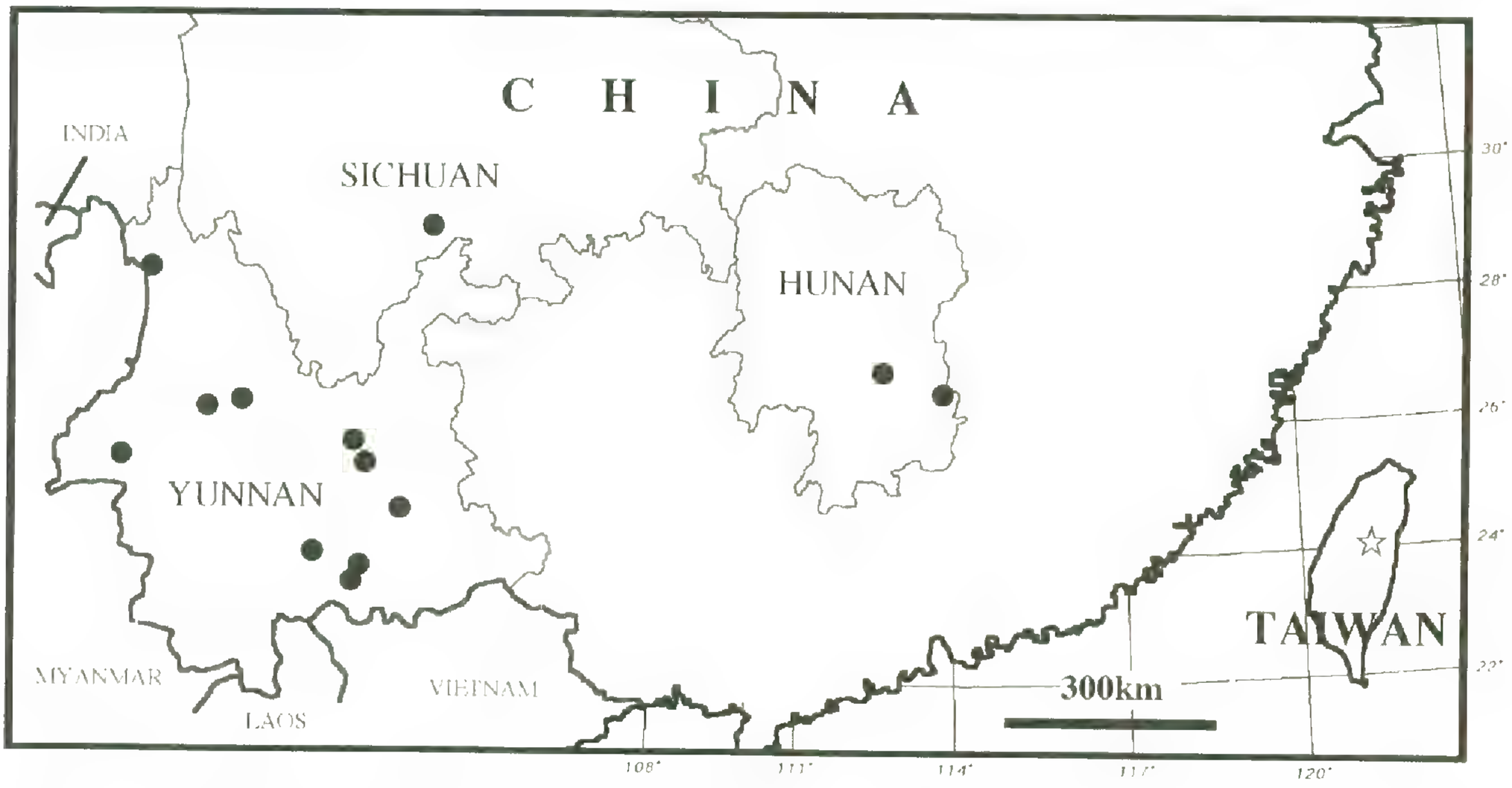


FIG. 2. Geographical distribution of *Deparia longipes*. The dots represent localities for *D. longipes* reported by Ching (1964) and Chu (1999), the star shows the new locality of *D. longipes* found in the present study.

Deparia. A molecular phylogenetic tree based on the nucleotide sequences of the *rbcL* gene made by Sano *et al.* (Mol. Phylog. Evol. 15: 403–413. 2000) revealed the monophyly of the four sections of the genus *Deparia* sensu Kato (1977; 1984), whereas interspecific relationships within *Athyriopsis* sensu Ching were not well resolved. Therefore, in agreement with Kato (1977; 1984), we transfer *A. longipes* from *Athyriopsis* to *Deparia* sensu Kato, as indicated below.

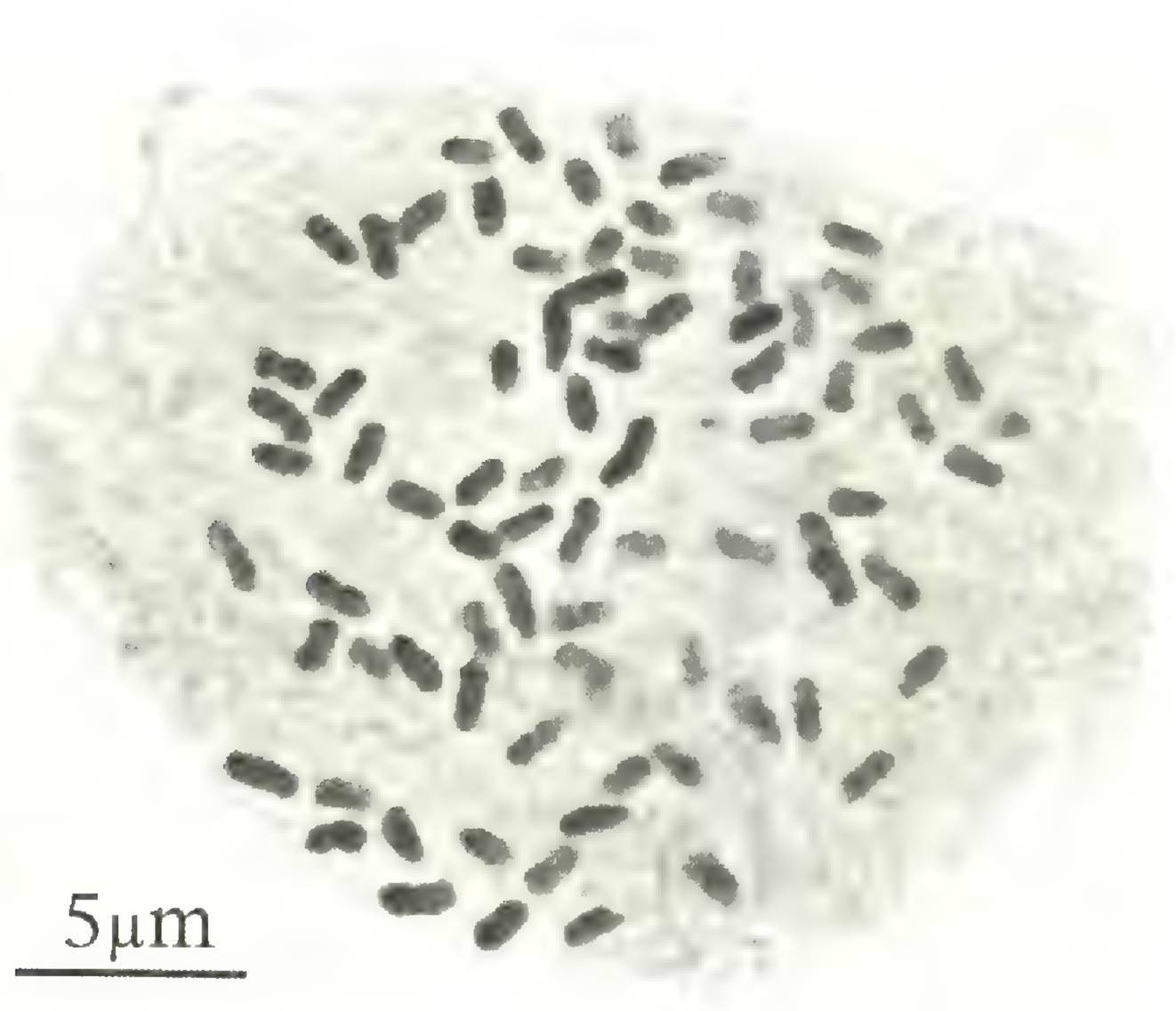


FIG. 3. Somatic chromosomes of *Deparia longipes* from Taiwan, showing 2n = 80.

Deparia longipes (Ching) Shinohara, ***comb. nov.***

Basionym: *Athyriopsis longipes* Ching, Acta Phytotax. Sin. 9(1):68 (1964). Type – China: Kunming, Sih-shan, Hua-ting-tze, 6 July 1945, *T. N. Lion 14278* (holotype: PE!)

This species belongs to *Deparia* based on the shallow rachis groove, which does not open at the junctions with the costae (Fig. 1–B).

Most species in sect. *Athyriopsis* sensu Kato (1977; 1984) are tetraploid or hexaploid. Diploids are known only in *D. petersenii* from Taiwan (Shinohara *et al.*, Int. J. Plant Sci. 167(2): 299–309. 2006). Polyploids are derived from diploids, and therefore information on ancestral diploid cytotypes is indispensable for understanding the origin of polyploids. The diploid cytotype of *D. longipes* found in the present study is, therefore, important for further biosystematic study of this group of ferns.

The authors would like to thank the curators of the Herbarium, at the Institute of Botany, Academic Sinica, Beijing (PE), and the Herbarium of Yunnan University (PYU).—Wataru Shinohara, Department of Botany, Graduate School of Science, Kyoto University, Kyoto, 606-8502, Japan, Tsai-Wen Hsu, Department of Botany, Taiwan Endemic Species Research Institute, Nantou 552, Taiwan, Shann-Jye Moore, Department of Life Science, National Taiwan Normal University, 88 Sec 4, Ting-Chow Rd, Taipei 116, Taiwan, Ho-Ming Chang, Department of Botany, Taiwan Endemic Species Research Institute, Nantou 552, Taiwan, and Noriaki Murakami, Makino Herbarium, Tokyo Metropolitan University, Hachioji, Tokyo, 192-0397, Japan.

American Fern Journal 96(3):100–102 (2006)

Review

Ferns of Northeastern and Central North America, 2nd ed., by Boughton Cobb, Elizabeth Farnsworth and Cheryl Lowe. 2005. [Peterson Field Guide Series] Houghton Mifflin Co., Boston. 417 pp. $20.00.

After much too long a wait, Boughton Cobb's guide to ferns is once again available. Elizabeth Farnsworth and Cheryl Lowe are to be congratulated for rewriting, with the assistance of a host of area specialists from across North America, this new version of a familiar favorite. Species coverage in the new addition is expanded and updated, taking advantage of new discoveries and new understandings within the field of pteridology. Each taxon entry begins with nomenclature and common synonymy followed by sections on habit, ecology and range. The greatly amplified morphological characterizations that follow are broken down by character and emphasize distinctive features. Treatments generally conclude with a short, concise 'notes' section that covers especially diagnostic features, similar species and, in the case of well-understood polyploids, ancestral taxa. All in all, this second edition fulfills the presumed goal of providing an update of Cobb's original field guide.

This new *guide* is a fancier, glossier version of the original with new sketches, by co-author Elizabeth Farnsworth, to supplement the elegant simplicity of Laura Louise Fisher's original artwork. It is unfortunate that some of these newer drawings did not reproduce well and appear rather coarser than Fisher's plates. The line art is, however, reinforced with 105 illustrative color photos as well as numerous others added for art's sake. With few exceptions these photos add greatly to an appreciation of form and characteristics of the plants being illustrated.

This field guide, while a wonderful and artistic production, is not without problems. Most seriously, in my opinion, is that it suffers from a certain amount of 'unevenness' in content, format and organization. Color plates appear outside of species treatments, either with the generic treatment or following full species treatments, on those pages reserved for 'secondary species'. I get a sense that their use within the book is more opportunistic than planned: *Dryopteris* with 13 taxa has four color plates; the monotypic *Onoclea*, *Marsilea* and *Deparia* each have 2 plates apiece; and several genera, *Azolla*, *Pleopeltis*, *Pteridium*, *Schizaea*, *Ophioglossum*, *Lycopodiella*, *Psuedolycopodiella* and *Isoetes*, have no photographic plates at all. Such organizational problems are not unique to the illustrations. As part of the introductory section, for example, there is a rather nice, 5-page, illustrated introduction to fern morphology. This section, however, is not integrated with the sometimes redundant, 7-page glossary at the end of the book. In this same vein, the introductory section "Fern Habitats and Conservation" is separated from the topically associated "Ferns in Cultivation and Culture" section at the back of the book.

More problematic perhaps is the uneven species treatments found in many genera. As an example, the *Adiantum* treatment, covering four species, consists of two full pages for *Adiantum pedatum* and two interrupted pages for the remaining three. Admittedly those three species are rare, but perhaps no more so than *Asplenium scolopendrium*, with two full pages of coverage. Similar discrepancies in treatment can be found in other genera. Taxonomic inequity is a problem at the generic level as well, although here it is a matter of circumscription rather than coverage. This is seen especially clearly toward the end of the book where the classic genus *Lycopodium* is split, not into three genera as in many modern treatments such as the *Flora of North America*, but into seven, including the most recent segregates *Dendrolycopodium* and *Spinulum*. Morphological and molecular data sets are available that clearly show that these splinter genera are monophyletic, but the ability to split does not translate into the requirement to do so. Current trends to treat all monophyletic groups at the generic or familial rank, while transmitting a certain set of phylogenetic information, completely ignore phylogenetic relationships at other hierarchical levels. With the generic concept in the new edition, we lose sight of the group relationships among *Diphasiastrum*, *Lycopodium*, *Spinulum* and *Dendrolycopodium* and between *Lycopodiella* and *Pseudolycopodiella*. The stated argument for segregate genera is far from compelling: "These relatively new generic names [*Spinulum* and *Dendrolycopodium*] may not yet be familiar to many botanists, but we expect they will be more common in the next few years." The splitting evidenced in the lycopod section is in direct contrast to the situation in the Ophioglossaceae and Equisetaceae. Both of these families contain morphologically and molecularly distinct clades that are not recognized at the generic level. Instead, the genus *Botrychium* is maintained as a whole with the caveat "...in keeping with the *Flora of North America* (1993) and the majority of current treatments, we retain the species under the single genus *Botrychium*...." This lack of consistency is a problem; unnecessary splitting of genera does not help young fern students establish an understanding of rank or a feeling for groups and their relationships.

The arrangement of genera in the new edition is puzzling. The *"True Ferns"* are divided into the higher leptosporangiate ferns (+ *Osmunda*) and the succulent ferns (*Botrychium* and *Ophioglossum*). Within these two headings the genera are arranged alphabetically. The next section, *"Fern Relatives"*, is four parted and a bit more quixotic in arrangement. Part one consists of the lycopods – all those species (and genera) previously treated as *Lycopodium* in the first edition. *Equisetum*, *Isoëtes*, and *Selaginella* follow sequentially. This anomalous admixture of alphabetizing and phylogeny is discordant and misleading. On pages 11-13, the authors discuss higher-level fern relationships and clearly show the inclusion of *Equisetum* within the monilophytes; that relationship is ignored within the organizational framework of the book. Family level relationships are essentially lacking. Only occasionally are family names mentioned and so again the beginner is at a loss for organizational tools as they attempt to understand and make sense of fern diversity.

My final concern with the book is that it fails to provide a solid stepping-stone for more advanced studies of difficult fern groups. One aspect of fern biology that is downplayed is the frequency and evolutionary importance of hybridization. For serious fern students, the search for hybrid taxa in genera such as *Dryopteris*, *Cystopteris* and *Asplenium* can represent an exciting, challenging, and educational experience. The *guide's* glossary definition of "hybrid" is clear, but does not make an adequate distinction between primary hybrids and hybrid derived taxa, both of which may be important elements in fern rich areas within the area covered. In fact, as one examines species treatments across genera in which hybridization does occur, one notes that typically all of the higher ploidy taxa are called *hybrids*, and often given less coverage as a result. Such indiscriminate use of the term hybrid is confusing to the amateur and the unwary and misrepresents the actual biology of the organisms concerned. Although two (of the stated 27) hybrids are enumerated under *Dryopteris* they are provided with short, inconsequential descriptions (on the same page as two geographically peripheral fertile taxa) and are poorly, if at all, illustrated. There is no mention of other common hybrids, for example those involving *Dryopteris marginalis*, and no help provided as to how to know when one has found a hybrid. Space is of course always an issue with such field guides and the authors were undoubtedly constrained by page length.

My very first fern book was *A Field Guide to the Ferns* written by Boughton Cobb and published by Houghton Mifflin Company in 1956. My copy is from the seventh printing and as I examine this well worn book I notice years of notes, locality data, dates of my first finds of a species, and even a snippet of *Cheilanthes tomentosa* as a reminder of an exciting day in the field with Herb Wagner and Joe Beitel. I no longer use Cobb's book as the doorway into a new and exciting world, but I do use it as a reminder of youthful exuberance and exploration. Now, as new generations of burgeoning pteridologists begin their careers, they may also carry a copy of Cobb with them into the field and carefully annotate their discoveries in the pages of this new and expanded version. Although I have some disagreements with coverage and scope, I have already bought and given away several copies of this book to young fern students and I heartily recommend it as *the* starting point for young fern enthusiasts of northeastern North America.—R. James Hickey, Botany Department, Miami University, Oxford, OH 45056.

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerox copies acceptable), including review copies of illustrations and originals of illustrations. After review, submission of final versions of manuscripts on diskette (in PC- or Mac-compatible formats) is strongly encouraged. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, short, informative abstract, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Do not submit right-justified copy, avoid footnotes, and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence et al., 1968, Hunt Botanical Library, Pittsburgh) and its supplement (1991). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, omit the abstract and put all references parenthetically in text. Use *Index Herbariorum* (Regnum Veg. 120:1–693. 1990) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matter of form or style, consult recent issues of *American Fern Journal* and *The Chicago Manual of Style*, 14th ed. (1993, Univ. Chicago Press, Chicago). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

PTERIDOLOGIA ISSUES IN PRINT

The following issues of *Pteridologia*, the memoir series of the American Fern Society, are available for purchase:

1. Wagner, David H. 1979. Systematics of Polystichum in Western North America North of Mexico. 64 pp. $10.00 postpaid.

2A. Lellinger, David B. 1989. The Ferns and Fern-allies of Costa Rica, Panama, and the Chocó (Part 1: Psilotaceae through Dicksoniaceae). 364 pp. $32.00 postpaid.

3. Lellinger, David B. 2002. A Modern Multilingual Glossary for Taxonomic Pteridology. 263 pp. $28.00 postpaid.

Send your order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

AMERICAN FERN JOURNAL ON MICROFICHE

Volumes 1–61 of the *American Fern Journal* are available as archival quality, silver positive microfiches. Single volumes or the entire run may be purchased. The fiches are easily read with 10x or greater magnification (using a dissecting microscope and transmitted illumination or a fiche reader). Silver negative microfiches of vols. 1–50 are also available. The price is $4.00 per volume or $244.00 per set of 61 volumes, postpaid.

Send your inquiry or order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

FIDDLEHEAD FORUM

The editor of the Bulletin of the American Fern Society welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

SPORE EXCHANGE

Ms. Denia Mandt, 12616 Ibbetson Ave., Downey, CA 90242-5050, is Director. Spores exchanged and lists of available spores sent on request. http://amerfernsoc.org/sporexy.html

GIFTS AND BEQUESTS

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Back issues of the Journal and cash or other gifts are always welcomed and are tax-deductible. Inquiries should be addressed to the Secretary.

VISIT THE AMERICAN FERN SOCIETY'S WORLD WIDE WEB HOMEPAGE: http://www.amerfernsoc.org/

AMERICAN FERN JOURNAL

Volume 96

Number 4

October–December 2006

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Growth rates and age estimates of *Alsophila setosa* Kaulf. in Southern Brazil
Jairo L. Schmitt and Paulo G. Windisch **103**

***Thelypteris sancta* (L.) Ching, New for Florida and the Continental United States**
Keith A. Bradley **112**

Gametophyte Morphology in Three Mexican Species of *Bolbitis* (Lomariopsidaceae)
Blanca Pérez-García and Aniceto Mendoza-Ruiz **115**

Characterization of a *Thelypteris* Hybrid from Walker County, Texas
Tracy E. Willis and Joan E. Nester-Hudson **127**

SHORTER NOTES

Chemotaxonomic survey of flavonoids from *Sphaerostephanos* (Thelypteridaceae) of Peninsular Malaysia
Umikalsom Yusuf, Nancy Martina Jacob, Mohd Aspollah Sukari, and Khairuddin Itam **134**

Referees for 2006 **139**

Index to Volume 96 (2006) **140**

The American Fern Society

Council for 2006

DAVID S. CONANT, Dept. of Natural Sciences, Lyndon State College, Lyndonville, VT 05851.
President

WARREN D. HOAK, Dept. of Biological Sciences, Denison University, Granville, OH 43023
President-Elect

W. CARL TAYLOR, 800 W. Wells St., Milwaukee Public Museum, Milwaukee, WI 53233-1478.
Secretary

JAMES D. CAPONETTI, Dept. of Botany, University of Tennessee, Knoxville, TN 37916-1110.
Treasurer

GEORGE YATSKIEVYCH, Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299.
Membership Secretary

JAMES D. MONTGOMERY, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.
Curator of Publications

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Journal Editor

R. JAMES HICKEY, Botany Dept., Miami University, Oxford, OH 45056.
Memoir Editor

ROBIN HALLEY, 1418 Park Row, La Jolla, CA 92037-3710
DAVID SCHWARTZ, 9715 Christey Way, Bakersfield, CA 93312-5617
Bulletin Editors

American Fern Journal

EDITOR

R. JAMES HICKEY ..... Botany Department, Miami University, Oxford, OH 45056,
ph. (513) 529-6000, e-mail: hickeyrj@muohio.edu

ASSOCIATE EDITORS

GERALD J. GASTONY ..... Dept. of Biology, Indiana University, Bloomington, IN 47405-6801
GARY K. GREER ..... Biology Dept., Grand Valley State University, Allendale, MI 49401
CHRISTOPHER H. HAUFLER ..... Dept. of Ecology and Evolutionary Biology, University of Kansas, Lawrence, KS 66045-2106
ROBBIN C. MORAN ..... New York Botanical Garden, Bronx, NY 10458-5126
JAMES H. PECK ..... Dept. of Biology, University of Arkansas–Little Rock, 2801 S. University Ave., Little Rock, AR 72204

The "American Fern Journal" (ISSN 0002-8444) is an illustrated quarterly devoted to the general study of ferns. It is owned by the American Fern Society, and published at The American Fern Society, % Missouri Botanical Garden, P. O. Box 299, St. Louis, MO 63166-0299. Periodicals postage paid at St. Louis, MO, and additional entry.

Claims for missing issues, made 6 months (domestic) to 12 months (foreign) after the date of issue, and orders for back issues should be addressed to Dr. James D. Montgomery, Ecology III, 804 Salem Blvd., Berwick, PA 18603-9801.

Changes of address, dues, and applications for membership should be sent to the Membership Secretary.

General inquiries concerning ferns should be addressed to the Secretary.

Back volumes are avalible for most years as printed issues or on microfiche. Please contact the Back Issues Curator for prices and availability.

Subscriptions:

Society Membership – USA, Canada, Mexico (includes Journal and Fiddlehead Forum)	$25
Society Membership – All other countries (includes Journal and Fiddlehead Forum)	$32
Society Life Membership – $300 (add $140 mailing surcharge for outside USA, Canada, Mexico)	
Regular Membership – USA, Canada, Mexico (includes Fiddlehead Forum)	$12
Regular Membership – All other countries (includes Fiddlehead Forum)	$19
Institutional Membership – $35 to USA, Canada, Mexico; $45 elsewhere (-$2 agency fee)	

POSTMASTER: Send address changes to American Fern Journal, Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166-0299.

American Fern Journal 96(4):103–111 (2006)

Growth Rates and Age Estimates of *Alsophila setosa* Kaulf. in Southern Brazil

JAIRO L. SCHMITT
Centro Universitário FEEVALE, 93510-250 Novo Hamburgo, RS, Brazil
PAULO G. WINDISCH
Universidade do Vale do Rio dos Sinos, UNISINOS, PPG-BIOLOGIA,
93022-000 São Leopoldo, RS, Brazil

ABSTRACT.—The tree fern *Alsophila setosa*, occurs in primary and secondary forests of southern and southeastern Brazil. Two populations in secondary forest formations in the northeastern part of the State of Rio Grande do Sul, in the municipalities of Morro Reuter (45 plants) and Sapiranga (48 plants), were studied to estimate the ages of the plants. Two approaches were tested, one based on the total length in relation to the yearly growth rate of the caudex, and the second on the total number of scars and remains of stipe bases along the caudex in relation to the yearly frond production. Estimates based on growth rates and total length did not agree with the information and records of the past land use, whereas frond production over a longer time period presented acceptable estimates. The development of a new plant formed through vegetative reproduction was observed during three years. A brief discussion of the problems of age estimates in tree ferns is presented.

Biological data are urgently needed for conservation efforts of endangered species such as tree ferns subject to commercial exploitation. Some species are the source of fibrous material formed by the adventitious root mass covering the caudex which is used for growing orchids and aroids, while others have plants removed from the field and used in landscaping and decoration. Even with adherence to the CITES agreements restricting the trade, local use still represents a major pressure for conservation. Forest management plans tend to exclude tree ferns or inflate the information on their growth and regeneration potential, while the concerned protection agencies lack proper information on their growth rates. A better understanding of the role of tree ferns and the presence of some epiphytic species is still urgently needed. In some cases, as discussed by Coomes *et al.* (2005), tree ferns offer conditions for the germination and initial establishment of some woody vascular seed plants, thus having an important role in their regeneration.

Tree ferns present some interesting biological aspects, growing many meters tall, sometimes up to the forest canopy, while presenting only primary tissue and a water supply through tracheids. Their growth, compared to some woody plants can be considered slow, but the lack of more precise field data constitutes a major problem in establishing the age of individual plants. Ages of tree ferns tend to be estimated using a) the total caudex length in relation to the yearly growth rates or b) the yearly frond production in relation to the total number of frond vestiges (old fronds, remains of stipe bases or scars) along the caudex. The first method assumes that the growth is constant throughout the

life-span, while the initial stages, from the formation of the young sporophyte on the gametophyte are left out from the estimates. The second method cannot be applied to all arborescent species without serious damage to the plants (leaf scars covered by adventitious roots and/or remains of stipe bases) while the initial development, up to plants presenting a definite caudex with leaf scars, is also left out of the estimates.

Age estimates based on the total caudex length and yearly growth rates were presented by Conant (1976) for Cyathea *arborea* (L.) Sm. and *Alsophila bryophila* R. M. Tryon, growing in Puerto Rico; Ortega (1984) for *Sphaeropteris senilis* (Klotzsch.) R. M. Tryon, in Venezuela; Bittner & Breckle (1995) for *A. erinaceae* (H. Karst.) D. S. Conant, *A. polystichoides* (H. Christ) Domin, *C. delgadii* Sternb., *C. nigripes* (C. Chr.) Domin, *C. pinnula* (H. Christ) Domin and *C. trichiata* (Maxon) Domin, in Costa Rica.

Seiler (1981) estimated the age of *Alsophila salvinii* Hook., in El Salvador based on the frond production and the scars along the caudices, pointing out that the exact age could not be established as the frond production rate of very young plants is not known. Tanner (1983) using the same basic method estimated the age of *Cyathea pubescens* Mett. *ex* Kuhn plants growing in Jamaica. Ash (1987) estimated the age of *C. hornei* (Baker) Copel. plants in Fiji, including more information on the spacing of the frond scars.

Walker & Aplet (1994) presented age estimates and information on the growth response of the caudex of *Cibotium glaucum* (J. Sm) Hook. & Arn. after the addition of nutrients, noting that Nitrogen or Phosphorous increased the growth rate. These authors also verified that the caudex has higher growth rates in 1000 year old forest plots, when compared to that of younger forest formations. Durand and Goldstein (2001) evaluated relative growth rates and the reproductive potential in native (*Cibotium* spp.) as well as invasive (*Sphaeropteris cooperi* (Hook. *ex* F. Muell.) Tryon) tree ferns in Hawaii and verified that the invasive species presents a more intensive growth. Arens (2001) studied growth rates of *Cyathea caracasana* (Klotzsch.) Domin, at different sites in Colombia, under differing ecological conditions and distinct regeneration stages.

Among the tree ferns used for landscaping and decoration in the State of Rio Grande do Sul, Brazil, *Alsophila setosa* Kaulf. (Cyatheaceae) is currently being extracted from forest remnants (Windisch, 2002). The existing populations are also being reduced by deforestation for lumbering and use of the land for grazing or agriculture (Schmitt and Windisch, 2005). This species grows in primary and secondary humid forests in South and Southeastern Brazil, between 20 m and 1800 m elevation. In southern Brazil it occurs also in forests with *Araucaria* and *Podocarpus* as well as humid places in semi-deciduous forest formations. The plants grow up to 10 m tall, have a caudex ca. 10 cm in diameter (Gastony, 1973), with remains of stipe bases (with blackish spines) on the distal part and frond, and scars (from fallen fronds) on the basal portion. The crown is up to 3 m long and has fronds with tripinnate-pinnatisect laminae (adult plants). Considering the lack of biological data for *A. setosa*, as well as the need for more knowledge of tree fern development, growth rates

and comparative age estimations based on different approaches are being presented.

Material and Methods

Fieldwork was conducted in two semi-deciduous seasonal broadleaved forest formations in the northeastern part of the State of Rio Grande do Sul, Brazil. The first site is located within the municipality of Morro Reuter (29°32′ S, 51°04′ W, alt. 700 m) and consists of a forest remnant surrounding a spring that is still in use as a local water supply, being protected from cattle and from deforestation (confirmed by local residents). The area is surrounded by less homogeneous secondary growth. The second site, at the municipality of Sapiranga (29°38′ S, 51°00′ W, alt. 570 m), is situated on an abandoned field that was in agricultural use up to the early 1960s. An aerial photograph of the region shows that this particular area was devoid of tree cover as late as December of 1964, so that the current secondary forest stand at the time of the observations was less than 40 years old. The selection of two populations was made without intending comparative analyses between the two populations, but basically in order to guarantee results even if predatory extraction of ferns or lumbering resulted in the loss of one of the populations during the course of the study.

Basic soil analysis (NPK) of samples from both localities was performed at the Geosciences Laboratory of the Universidade do Vale do Rio dos Sinos. Voucher specimens were placed at the Universidade do Vale do Rio dos Sinos Herbarium (HASU 10176; HASU 9773).

The climate is humid sub-tropical (Teixeira *et al.*, 1986), and data from the nearest meteorological station (Ivoti, 127 m alt) indicate an average mean temperature of the coolest month of 13.3°C (July), average of the warmest month of 25.1°C (February), absolute minimum −1°C (July) and absolute maximum 35.7°C (September and January) during the study period. Total precipitation was 2138 mm, the driest month being May (40.9 mm in 2000 and 78 mm in 2001), with maximum rainfalls in September (232.2 mm), October (303.9 mm), and January (308.6 mm). Data for an extended period of time could not be obtained. Frost occurs several times during more intense winters.

Two approaches were used to estimate the age of the plants. One was based on the relationship between the average yearly growth rate in height and the total length of the caudex. The second was based on the relationship between the average number of fronds produced during the year in relation to the total number of fronds along the caudex, as determined by counting fronds and their vestiges (remains of stipes and scars left by the fronds after complete decomposition of the stipe bases).

For frond production analysis, plants up to 4 m tall (the maximum size that safely could be reached in order to follow the frond production at the apex) were marked by placing wooden stakes with aluminum labels in the ground close to the base of each caudex. Larger plants with caudices up to 4.7 m tall can be found in the sites, but were not studied. At Morro Reuter 45 plants were

marked and another 48 at Sapiranga. Each caudex with a crown of fronds was considered to be an individual. Caudex length was measured from the ground surface to the extreme apex at the center of the crown of fronds.

Initial field observations of frond production were conducted from May 2000 to April 2001, with the height of the caudices registered at the beginning and end of this period. Annual frond production was determined by marking (with a loose loop of synthetic fiber) the youngest expanding crozier on each marked plant at the beginning of the observation period, this serving as a reference for recognizing the new fronds produced during the study period.

In order to estimate the total numbers of fronds produced at each site, 10 individuals were sampled, leaving out plants with caudices less than 40 cm tall and taller than 3.5 m; the latter were mostly covered by adventitious roots or epiphytes making counting impossible. When a part of the caudex presented patches of epiphytic cover, the average between the preceding and the next 20 cm sections was used as an estimate of the number of scars present underneath the covered part.

In August 2003, additional data set were collected from nine caudices in each population, removing all the epiphytes in order to obtain a precise count of the total number of stipe bases and scars. This also allowed the verification of caudex growth in length and frond production over a three-year period. On that occasion, the development of a young plant, which in October 2000 was sprouting out of the ground close to one of the marked specimens, was evaluated. This new individual started out from a well developed crozier, emerging from the soil and clearly was not a small plantlet formed from a gametophyte.

Pearson's correlation test was used to evaluate the relationships between the length of the caudex and the total number of scars and stipe bases, the length of the caudex, and the yearly frond production. Linear regression analysis was used to estimate the number of stipe bases and the yearly frond production relative to caudex length. In the second approach, the age estimate resulted from the equation: total number of fronds and vestiges / yearly frond production rate.

Data analysis was performed as described by Vieira (1980) and Zar (1999) using the SPSS 9.0 program installed at the Universidade do Vale do Rio dos Sinos data processing facility.

Results

During the 12 month period the average caudex growth rate based on the samples from Morro Reuter was 14.51 (s.d. = ±11.49) $cm.yr^{-1}$, ranging from a maximum rate of 47 $cm.yr^{-1}$ to three individuals with no noticeable growth as (although new fronds were formed). In Sapiranga the average growth rate was 6.32 (s.d. = ±5.53) $cm.yr^{-1}$, with a maximum growth of 22 $cm.yr^{-1}$ and ten plants showing no noticeable length increase. The growth rates of these populations were significantly different ($P < 0.001$). There was no significant

TABLE 1. Comparative age estimates of an *Alsophila setosa* plant with a 4 m long caudex, using the one year data (n = 45 for Morro Reuter, n = 48 for Sapiranga).

Locality	Growth rate	Estimated age based on growth rate	Age based on frond production equation
Morro Reuter	14.51 cm.yr^{-1}	27 years	22 years
Sapiranga	6.32 cm.yr^{-1}	63 years	32 years

correlation between caudex growth and total length in Morro Reuter ($r = 0.258$, $P = 0.086$, $n = 45$) or in Sapiranga ($r = 0.166$, $P = 0.253$, $n = 48$).

A strong positive correlation between the length of the caudex and the number of scars and stipe bases was observed both in Morro Reuter ($r = 0.856$, $P = 0.02$, $n = 10$) and in Sapiranga ($r = 0.825$, $P = 0.03$, $n = 10$). The yearly frond production also showed a strong positive correlation with caudex length both in Morro Reuter ($r = 0.845$, $P = 0.02$, $n = 10$) and Sapiranga ($r = 0.876$, $P = 0.01$, $n = 10$). Based on these strong direct correlations, a linear regression analysis was performed in order to obtain equations to estimate the number of scars per total caudex length and to predict yearly frond production. The number of scars/stipe bases in relation to the length of the caudex (H), in Morro Reuter was estimated as: $y = -12.115 + 0.9H$ and the yearly frond production by $y = -0.773 + 0.042H$. In Sapiranga the equations were respectively $y = 47.149 + 0.577H$ and $y = -0.0138 + 0.0218H$.

Approximate age estimates for hypothetical plants with 4 m long caudices using the first approach (total caudex length divided by the average growth rates) are presented in Table 1. In the case of the Sapiranga site, the resulting age estimate does not agree with the age of the forest.

For estimating age based on the total number of fronds in relation to the yearly frond production, for Morro Reuter plants the formula would be $(-12.115 + 0.9\ H)/(-0.773 + 0.042H)$, whereas for Sapiranga it would be $(47.149 + 0.577H)/(-0.0138 + 0.0218H)$. These results indicated lower ages than those using the first approach (Table 1).

The analysis of the preliminary data indicated that a bias was introduced by the variation in growth pattern during the initial phases of development. Also the frond production rate in a particular year does not reflect the average growth conditions throughout the life span of a given plant. This led to a new survey of the marked plants still standing in the localities in August 2003. Data for caudex length and number of scar/stipe bases were obtained for a three year period for nine plants in each population. The sampled plants in Morro Reuter produced an average 8.66 fronds.yr^{-1} while those from Sapiranga 6.92 fronds.yr^{-1}. Age estimates based on these data for 4 m tall hypothetical plants are shown in Table 2.

As to the basic nutrients in the soil, no significant differences in NPK were found between populations based on t-tests (Nitrogen: $P = 0.981$, Phosphorous: $P = 0.440$ and Potassium: $P = 0.282$). Samples within each site did show heterogeneity among collecting points.

TABLE 2. Age estimates using the total number of fronds produced in two plants with 4 m tall caudices and the average (n = 9 in each population) yearly frond production (3 year period).

Locality	Scars/stipes in a 4 m long caudex	Average yearly frond production (3 years)	Estimated age
Morro Reuter	244	8.66 fronds·yr^{-1}	28 years
Sapiranga	238	6.92 fronds·yr^{-1}	34 years

The rapid growth of vegetatively produced plants is illustrated by the single individual, initially observed in October 2000 at the Morro Reuter site, represented by a crozier emerging from the ground. In February 2001 its caudex was 7 cm. By April it had reached 10 cm and bore a crown of ca 3.5 m diameter with fronds up to 230 cm long. By September 2003, this plant had a 70 cm long caudex.

DISCUSSION

The average growth rates observed for *Alsophila setosa* are within the range of growth rates reported by other authors for other species of the genus (Table 3). The "no noticeable" growth status of some plants with reduced caudex growth may be due to the presence of unexposed young croziers (still covered by a mass of scales) at the tip of the caudex at the time of the first measurement. The differences in the growth rates between sites, in the present study, could be related to different canopies and different successional stages of the surrounding vegetation. Arens (2001) presented some interesting data on *Cyathea caracasana*, growing in different habitats in Colombia, indicating a life history with periods of rapid growth, spore production, and establishment in forest gaps, alternating with low growth rates and persistence in the understory, and recorded higher growth rates in open habitats.

Slightly lower growth rates were observed in Sapiranga, where the forest is younger and the upper canopy more continuous. *Alsophila setosa* is an understory plant, so that the height of the canopy seems to influence the height increase once the fronds reach that level. In our experience, this species never

TABLE 3. Growth of the caudex in different species of the genus *Alsophila*.

Species	Growth rate (cm·yr^{-1})	Locality	Forest	Source
Alsophila bryophila	5.0	Porto Rico	Primary	Conant (1976)
Alsophila salvinii	6.9	El Salvador	Secondary	Seiler (1995)
Alsophila erinacea	13.6	Costa Rica	Primary	Bittner & Breckle (1995)
Alsophila polystichoides	18.8	Costa Rica	Primary	Bittner & Breckle (1995)
Alsophila setosa	14.51	Brazil	Secondary	Present study
Alsophila setosa	6.32	Brazil	Secondary	Present study

surpasses the upper tree canopy. Also different heights of the crown of fronds may lead to differences in exposure to low temperatures. Frost damage to young croziers has been reported by Schmitt and Windisch (2001). Although the macro-nutrients (N, P, K) did not show significant differences, considering the different histories of soil usage in both plots, differences in micronutrients may occur between the study sites.

Considering the aerial photograph taken in 1964 of the area in Sapiranga site, the estimate of 32–34 years for the taller plants is much more realistic that the one obtained only using the yearly growth rate (63 years). The use of the frond production data, especially if obtained over a longer period of time, produces acceptable values (compare Tables 1 and 2). This would mean that the tallest plant at the locality (4.7 m) would be about the same age as the forest stand.

However, using the formulas applied in the example in Table 1 to a plant taller than 4 m the resulting age would have lower value than the one obtained for a 4 m plant, as the sample includes a larger number of younger plants with different growth patterns. Attempts to exclude this bias were not successful due the size of the samples in the different age brackets of the studied plants, and surely also due to differences in their initial stages of development. Tanner (1983) indicated that the method using total number of fronds in relation to the yearly frond production gives more realistic values for growth in plants of lower stature, but less realistic values for caudices greater than 1 m tall.

Two of the variables affecting these estimates is the time needed to establish the sporophyte from spore and the growth up to the stage where a caudex with noticeable scars is formed and preserved throughout the life of the plant. Another variable is the reproductive origin of the plants. In species such as *Alsophila setosa*, with both vegetative and sexual reproduction, age estimates may differ for stems produced by the two reproductive modes. The rapid growth of the vegetatively formed individual is striking and indicates a strong potential for leaving the shaded ground cover by the rapid development in the first stages of these plants.

Based on the yearly frond production rates and the total frond vestiges on the caudex, Seiler (1981) estimated the age of 4.6 m tall *Alsophila salvinii* sporophytes at 55 years; Tanner (1983) estimated *Cyathea pubescens* with 9 m tall plants at 150 years old; Ash (1987) estimated 5.5 m tall plants of *C. hornei* as being between 80 and 105 years. The following age estimates based on yearly growth rates and total length of the caudices may be found in the literature: *Alsophila bryophyla*, 5 m tall, 5 $cm.yr^{-1}$ growth in length, considered to be 100 years old; *Cyathea arborea*, 5 m, 28.6 $cm.yr^{-1}$, 18 years (Conant,1976); *Sphaeropteris senilis*, 2 m, 4 $cm.yr^{-1}$, 50 years (Ortega, 1984); *A. erinacea*, 5 m, 13.6 $cm.yr^{-1}$, 37 years; and *A. polystichoides*, 5 m, 18.8 $cm.yr^{-1}$, 27 years (Bittner and Breckle, 1995).

There is a lack of field data on the first stages of gametophytic and sporophytic development for these species resulting in a poor understanding of the complete life cycle. The extraction of plants for cultivation represents the removal of material that may take decades to be formed, and the original

growth conditions for gametophytes and young sporophytes may no longer be available in the site. However, in species with vegetative propagation, such as *Alsophila setosa*, new individuals produced in this manner may occupy the available niches and perpetuate the presence of the species. These young individuals plants after a rapid growth through the herbaceous layer, become part of the stand, and many of the smaller plants considered in the present study probably had this origin. This presents additional problems for age estimates because we may not be differentiating between sporophytes of different origins and different growth characteristics.

Our data suggest that some of the previously published age estimates should be reconsidered, taking into account the different methods and problems outlined above. Longer observation periods, especially in areas of less uniform climates are highly desirable. In general, our growth rate results are similar to those for other species of tree ferns in different regions, especially those of *Cyathea caracasana* (Arens, 2001) *Alsophila bryophila* (Conant, 1976), *A. salvinii*, *A. erinacea* and *A. polystichoides* (Bittner and Breckle, 1995).

Acknowledgments

The support and facilities provided by Universidade do Vale do Rio dos Sinos - UNISINOS, Centro Universitário FEEVALE, the Brazilian Research Council – Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq made this study possible. Lucas Schmitt and Cristina Schmitt provided welcome help in the field. Anonymous reviewers provided stimulating suggestions for the improvement of the manuscript.

Literature Cited

Arens, N. C. 2001. Variation in performance of the tree fern *Cyathea caracasana* (Cyatheaceae) across a sucessional mosaic in Andean cloud forest. Amer. J. Bot. 88:545–551.

Ash, J. 1987. Demography of *Cyathea hornei* (Cyatheaceae), a tropical tree-fern in Fiji. Austral. J. Bot. 35:331–342.

Bittner, J. and S. W. Breckle. 1995. The growth rate and age of tree fern trunks in relation to habitats. Amer. Fern J. 85:37–42.

Conant, D. S. 1976. Ecogeographic and systematic studies in American Cyatheaceae. Ph.D. Thesis, Harvard University, Cambridge, Mass.

Coomes, D. A., R. B. Allen, W. A. Bentley, L. E. Burrows, C. D. Canham, L. Fagam, D. M. Forsyth, A. Gaxiola-Alcantar, R. L. Parfitt, W. A. Ruscoe, D. A. Wardle, D. J. Wilson and E. F. Wright. 2005. The hare, the tortoise and the crocodile: the ecology of angiosperm dominance, conifer persistence and fern filtering. J. Ecology 93:918–935.

Durand, L. Z. and G. Goldstein. 2001. Growth, leaf characteristics, and spore production in native and invasive tree ferns in Hawaii. Amer. Fern J. 91:25–35.

Gastony, G. J. 1973. A revision of the fern genus *Nephelea*. Contr. Gray Herb. 203:81–148.

Ortega, F. 1984. Notas sobre la autoecología de *Sphaeropteris senilis* (KL) Tryon (Cyatheaceae) en el Parque Nacional el Avila – Venezuela. Pittieria 12:31–53.

Schmitt, J. L. and P. G. Windisch. 2001. Prejuízos causados pela geada no desenvolvimento de *Alsophila setosa* Kaulf. (Pteridophyta, Cyatheaceae). Revista de estudos / Centro Universitário Feevale 24:79–88.

Schmitt, J. L. and P. G. Windisch. 2005. Aspectos ecológicos de *Alsophila setosa* Kaulf. (Cyatheaceae, Pteridophyta) no sul do Brasil. Acta Bot. Bras. 19:861–867.

SEILER, R. L. 1981. Leaf turnover rates and natural history of the Central American tree fern *Alsophila salvinii*. Amer. Fern J. 71:75–81.

SEILER, R. L. 1995. Verification of estimated growth rates in the tree fern *Alsophila salvinii*. Amer. Fern J. 85:96–97.

TANNER, E. V. J. 1983. Leaf demography and growth of tree-fern *Cyathea pubescens* Mett. Ex Kuhn in Jamaica. Bot. J. Linn. Soc. 87:213–227.

TEIXEIRA, M. B., A. B. COURA NETO, U. PASTORE and A. L. R. RANGEL FILHO. 1986. Vegetação. Pp. 541–620, in *Levantamento De Recursos Naturais.Volume 33*. IBGE, Rio de Janeiro.

VIEIRA, S. 1980. *Introdução à Bioestatística*, 3ª ed. Campus, Rio de Janeiro.

WALKER, L. R. and G. H. APLET. 1994. Growth and fertilization responses of Hawaiian tree ferns. Biotropica 26:378–383.

WINDISCH, P. G. 2002. Fern conservation in Brazil. Fern Gaz. 16:295–300.

ZAR, J. H. 1999. *Bioestatistical analysis*, 4th edn. Prentice-Hall, Upper Saddle River.

American Fern Journal 96(4):112–114 (2006)

Thelypteris sancta (L.) Ching, New for Florida and the Continental United States

KEITH A. BRADLEY
The Institute for Regional Conservation, 22601 S.W. 152 Ave., Miami, FL 33170

ABSTRACT.—While compiling a plant inventory for a 20 acre forest fragment in Florida City, Miami-Dade County, Florida, on March 30, 2006, an unusual *Thelypteris* species was collected. The specimens did not correspond to any species reported from Florida (Wunderlin and Hansen, 2000). Using keys in Proctor (1989), Sánchez *et al.* (2006), and Morton (1963) it was tentatively identified as *T. sancta* (L.) Ching. A specimen was sent to Alan R. Smith, an expert on the genus, who confirmed this identification.

Thelypteris sancta is a widespread species occurring from the Greater and Lesser Antilles through Central America and northern South America (Proctor, 1989; Sánchez *et al.*, 2006). It is the second member of *Thelypteris* subgenus *Amauropelta* to be found in Florida. The other member, *T. resinifera* (Desv.) Proctor, occurs in central Florida in De Soto, Hillsborough, Pasco, and Polk Counties. Subgenus *Amauropelta* contains about 200 species (Smith, 1974), and section *Amauropelta* about 50 (Smith, 1974). The subgenus is distinguished from other *Thelypteris* in the Florida and Caribbean area by having free veins reaching the margin above the sinus, simple hairs, and sessile reddish glands on the abaxial surface of the blades (Smith, 1974; Proctor, 1989). *Thelypteris sancta* (Fig. 1) has pinnate pinnatifid blades with one or two pairs of free pinnules at the base of each pinna, whereas *T. resinifera* does not have free pinnules. The overall aspect of *T. sancta* is quite different from other *Thelypteris* species in southern Florida. Because *T. resinifera* does not occur in southern Florida, the only other species in southern Florida with an erect stem and primarily pinnate pinnatifid fronds is *T. patens* (Sw.) Small *ex* R.P. St. John (subgenus *Cyclosorus*). *Thelypteris patens*, however, is a much larger species with veins converging at the sinus (Proctor, 1989; Wunderlin and Hansen, 2000).

About 200 individuals of *Thelypteris sancta* were observed on the edges of solution holes in oolitic limestone substrate. Many age classes were seen, from juvenile sporophytes to adults, and even many dead specimens. Some plants were under a dense canopy of the exotic tree *Schinus terebinthifolius* Raddi. Most of the population was in a rockland hammock with a canopy dominated by native hardwoods, especially *Quercus virginiana* Mill. and *Ficus aurea* Nutt. Associated ferns included *T. dentata* (Forssk.) E. P. St. John, *T. kunthii* (Desv.) C. V. Morton, and *Anemia adiantifolia* (L.) Sw. A review of 1940 aerial photographs of the site showed that there has been significant habitat succession in the last 66 years. The area found to contain *T. sancta* was formerly a pine rockland savanna with a canopy of *Pinus elliottii* Engelm. var.

FIG. 1. Habit view of *Thelypteris sancta*, inset showing arrangement of sori.

densa Little & K.W. Dorman. Some old *Pinus* stumps still remain on the site, but the area has succeeded to a closed-canopy rockland hammock forest dominated by hardwoods due to fire suppression. This succession was probably accelerated by seed rain from a rockland hammock about 125 m to the east of the fern population. Most of this historical hammock has been cleared; a small remnant fragment was surveyed and was found not to contain *Thelypteris sancta*. The forest floor in the historical hammock lacks extensive amounts of exposed oolitic limestone in contrast to the area where *T. sancta* was found.

Thelypteris species are not typically cultivated, except by fern enthusiasts, and an internet search for *T. sancta* found no evidence of cultivation anywhere. The closest native occurrence to the Florida population is about 350 km away in Cuba (Sánchez et al., 2006). The species has probably gone undiscovered in Florida due to its rarity. Unlike most natural areas in Miami-Dade County, the site where it was found has been poorly surveyed by biologists because much of the site is of poor quality and difficult to access. *Thelypteris sancta* is probably a naturally occurring member of the Florida flora derived from wind-blown spores from Cuba or elsewhere in the Greater Antilles. Whether the arrival was in recent years or prior to European settlement in Florida is unknown.

Voucher: **Florida**. Miami-Dade County: Florida City. Navy Wells #23 Preserve. Just east of SW 182 Ave. at theoretical SW 358 St. A couple of hundred individuals in rockland hammock and under a canopy of *Schinus terebinthifolius* on oolitic limestone. Easting 551780, Northing 2813288 (UTM17N, WGS84). 30 March 2006, *Bradley* 2644 (FTG, NY, UC, USF).

Acknowledgments

I thank Eric Fleites of The Institute for Regional Conservation, who was with me at the time of the discovery, noticing the plants at the same time I did, Christina Casado-Acorn and Emilie Young of the Miami-Dade County Environmentally Endangered Lands Program for funding the plant inventory of Navy Wells #23 Preserve, Alan R. Smith of the University of California for confirming the identification of the species and making recommendations on the manuscript, Kirsten Hines of The Institute for Regional Conservation for review of the manuscript, an anonymous reviewer, and James Hickey of Miami University for valuable suggestions on the manuscript.

Literature Cited

Morton, C. V. 1963. Some West Indian species of *Thelypteris*. Amer. Fern J. 53:1–56.

Proctor, G. R. 1989. Ferns of Puerto Rico and the Virgin Islands. Mem. New York Bot. Gard. 53:1–389.

Sánchez, C., M. G. Caluff and L. Regalado. 2006. Thelypteridaceae. Fl. Rep. Cuba, Ser. A. Pl. Vasc. 11(13):1–124.

Smith, A. R. 1974. A revised classification of *Thelypteris* subgenus *Amauropelta*. Amer. Fern J. 64:83–95.

Wunderlin, R. P. and B. F. Hansen. 2000. *Flora of Florida, Volume 1*. The University Presses of Florida, Gainesville.

American Fern Journal 96(4):115–126 (2006)

Gametophyte Morphology in Three Mexican Species of *Bolbitis* (Lomariopsidaceae)

Blanca Pérez-García and Aniceto Mendoza-Ruiz
Departamento de Biología, Botánica Estructural y Sistemática Vegetal, Universidad Autónoma Metropolitana — Iztapalapa, Av. San Rafael Atlixco 186, Col. Vicentina, 09340, México, D. F.

Abstract.—The development of gametophytes in *Bolbitis bernoullii*, *B. portoricensis* and *B. umbrosa* (Lomariopsidaceae) is described and compared. Spores are monolete, ellipsoid with prominent winged perispore. Germination is of the *Vittaria* type and the prothallial development is of the *Drynaria* type. Collenchyma-like thickenings at the corners of the wing cells were not observed. Adult gametophytes are cordate with scarce marginal hairs near the shallow notch. Gametangia are of the common type found in homosporous leptosporangiate ferns. *Bolbitis portoricensis* develops the first leaf of the sporophyte after 6 to 8 months, with polocytic stomata. In *B. portoricensis*, vegetative propagation is present in old thalli 6 to 8 months after cultivation.

The genus *Bolbitis* is a terrestrial or hemiepiphyitic fern, with dimorphic leaves and simple or pinnate blades. Some species have laminar buds, and all have fertile leaves with acrostichoid sori, and reticulate venation with or without included veinlets. Its spores are monolete, non-chlorophyllous, and winged (Mickel and Smith, 2004; Moran, 1995; Tryon and Tryon, 1982; Tryon and Lugardon, 1991). The genus contains about 45 species, a third of which are found in the New World; seven occur in the southeastern part of Mexico. These seven species grow between 0–1300 masl in very wet rainforests. Except for *B. portoricensis* (Spreng.) Hennipman, all species are uncommon or rare in Mexico.

The gametophyte morphologies are known for several Indian species: *Bolbitis semicordata* (Moore) Ching, *B. quoyana* (Gaud.) Ching, *B. subcrenata* (Hook. et Grev.) Ching in C. Chr. (Nayar, 1960; Nayar and Kaur, 1964a, 1964b, 1965a, 1965b, 1965c, 1971); and for Old Word species such as *B. angustipinna* (Hayata) Ito, *B. heteroclita* (Presl) Ching and *B. repanda* (Bl.) Schott (Hennipman 1970, 1977). In general terms, the mature prothallus of *Bolbitis* is cordate-thalloid, wider than long, with a dense, 6 to 8-celled thick cushion, and wide flat wings. Collenchyma-like thickenings are present in the corners of wing cells of some Indian species.

Gametophytes of *Bolbitis* can be morphologically different. For instance, the prothallus in *B. repanda* is cordate, with pluricellular branched hairs and, when old, has a lobed thallus. In *B. cladorrhizans* (Spreng.) Ching, the gametophyte is glabrous and ribbon-shaped or lobed. Other species such as *B. heteroclita* have cordiform-spatulate prothalli, with unicellular, secretory and papillate hairs sparsely distributed along the margin of the adult gametophyte. In species such as *B. semicordata, B. quoyana* and *B. subcrenata*, the adult cordate thallus is usually branched but may be un-branched and has club-

TABLE 1. Collection data for materials used in current study.

Scientific name	Collection No. and date	Site location	Habitat elevation
B. bernoullii	*AMR-843* 20/04/2004	Veracruz; Mun. San Andrés Tuxtla, Plot 69 at Biological Station Los Tuxtlas, UNAM, between Laguna Azul and Laguna Escondida.	SAP 20 m asl
B. portoricensis	*AMR-618* 03/05/2002	Veracruz; Mun. Catemaco, Peninsula Moreno, in direction on the river, ca. 14–15 km after Coyame, towards Adolfo López Mateos.	SAP 60 m asl
	AMR-848 20/04/2004	Veracruz; Mun. San Andrés Tuxtla, Plot 69 at Biological Station Los Tuxtlas, UNAM, between Laguna Azul and Laguna Escondida.	SAP 20 m asl
	AMR-857 22/04/2004	Oaxaca; Mun. Valle Nacional, 1 Km before San Mateo Yetla, in the direction of Valle Nacional, coming from La Esperanza.	BMM 120 m asl
	BPG-1189 21/04/2004	Veracruz; Mun. Catemaco, 6.5 Km after Tebanca, in direction of Hidalgo.	Remains of SAP 390 m asl
B. umbrosa	*BPG-1185* 21/04/2004	Veracruz; Mun. San Andrés Tuxtla, Biological Station of Los Tuxtlas, UNAM.	SAP 150 m asl

shaped, multicellular, marginal hairs, 2 to 6 cells long, with slender stalks and a swollen anterior region.

One year old, or older, gametophytes of *Bolbitis repanda* are broadly cordate or elongate, with brown marginal rhizoids, and are abundantly covered with simple glandular hairs. The gametangia are of the advanced type, as in all Lomariopsidaceae. The morphological variation of the gametophytes is not taxonomically significant, and offers no evidence for determining affinities to other genera of the Lomariopsidaceae (Stokey and Atkinson, 1957; Atkinson, 1973).

This paper describes and compares the morphology and development of three species in Mexico: *Bolbitis bernoullii* (Kuhn. ex H. Christ) Ching, *B. portoricensis* and *B. umbrosa* (Liebm.) Ching.

MATERIALS AND METHODS

Spores were obtained from live plants collected from several sites in Mexico (Table 1). Vouchers are deposited at the Herbario Metropolitano Ramón Riba y Nava Esparza (UAMIZ). Fertile pinnae of different individuals were placed in paper bags until the spores were released. To remove sporangia, a mesh with pores 0.074 mm in diameter was used to sieve them. The spores of each species were then sown in five Petri dishes with Thompson solution, mineral salts, and agar, at an average density of 100–150 spores per cm^2 (Mendoza-Ruiz and Pérez-García, 2003). The Petri dishes were placed inside transparent plastic bags to stop contamination and dehydration and placed under artificial sun light (75 Watts day-light lamps), a 12 h light/darkness photoperiod, and

a temperature of 23–25°C. To determine photoblastism, two dishes of each species were placed in the dark.

Results

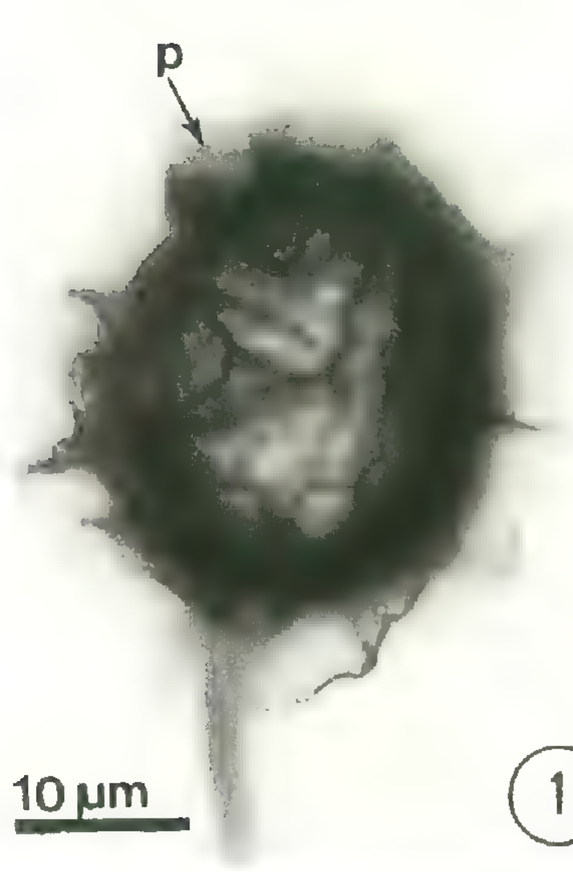
Spores of all three species are monolete, ellipsoid, flat-convex laterally, oblong in the extremes (poles), with a short leasura, and a smooth exine. *Bolbitis bernoullii* spores average 39(42)49 µm long × 31(35)39 µm wide, *Bolbitis umbrosa* 37(39)42 × 29(31)34 µm, and *B. portoricensis* 37(39)42 × 30(32)35 µm. In all three, the perine is prominent, membranous, thin, with wrinkled undulate folds, and psilate. In *Bolbitis bernoullii* the perine measures 6(7)10 µm, in *B. umbrosa* 5(7)10 µm, and in *B. portoricensis* 4(5)7 µm (Figs. 1, 2 and 3).

Once the spore germinates, the exine ruptures from the leasura and the first rhizoid, which is hyaline and has scarce plastids, emerges. The first prothallial cell develops inside the spore wall and contains numerous chloroplasts and yellow, oily globules (Fig. 4). Germination begins between days 8 and 9 in *Bolbitis portoricensis*, and between days 14 and 22 in *B. umbrosa* and *B. bernoullii*. Spore germination is of the *Vittaria*-type (Mendoza, 2001) and results in a proximal rhizoid and, lateral to it, a uniseriate germinal filament with 2 to 6 short, barrel-shaped cells, each wider than long and densely chlorophyllous. This early phase takes place between days 9 and 22 in *B. bernoullii, B. portoricensis* and *B. umbrosa*; development across species is asynchronous (Figs. 5–8). After 100 days, none of the spores kept in the dark germinated.

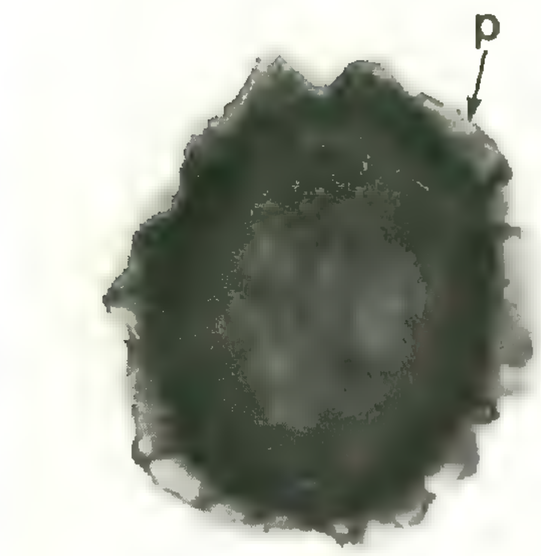
In *Bolbitis portoricensis* and *B. bernoullii*, the development of a prothallial lamina starts with repeated longitudinal and transverse divisions of the antepenultimate cells of the filament, and, with the expansion of the resulting daughter cells, gives rise to a 7- to 14-celled plate phase with an apical, obconic meristematic cell on day 22 (Figs. 9–10). Filaments in *B. umbrosa* are short and immediately undergo longitudinal and transverse divisions that initiate the 5- to 7-celled, short, wide and cordiform-looking plate phase on day 22; the obconic meristematic cell in these plates is not clearly defined.

The prothallial lamina of *Bolbitis portoricensis*, from day 16 to 38, is cordate-spatulate, with 20 to 120 cells, has a central pluricellular meristem, and is glabrous (Figs. 11–12). Rhizoids are located at the base of the gametophyte (Figs. 13–14). In *B. bernoullii* (Fig. 15) and *B. umbrosa*, the laminar phase is widely cordate, glabrous, of ca 60 to 70 cells, and has a centrally positioned meristematic zone in the shallow apical notch; rhizoids appear on day 37 and are located along the central part of the gametophyte.

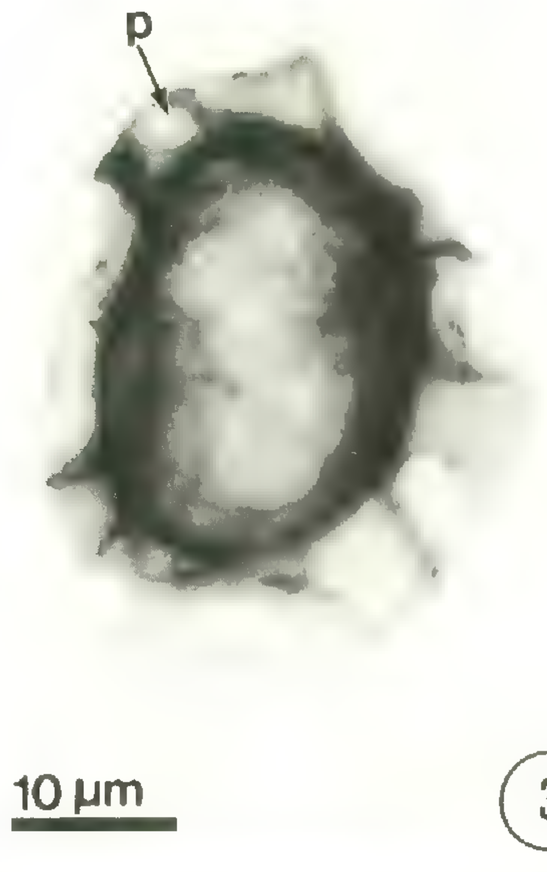
Prothallial development is of the *Drynaria*-type (Nayar and Kaur, 1969); the establishment of a meristematic cell occurs when the prothallial lamina is 7 to 14 cells wide. In some cases, a meristematic cell stage is absent and, a pluricellular meristem develops from the anterior marginal cells. A characteristic feature of this type of development is the much slower establishment of the apical meristem cell and the development of hairs on



1

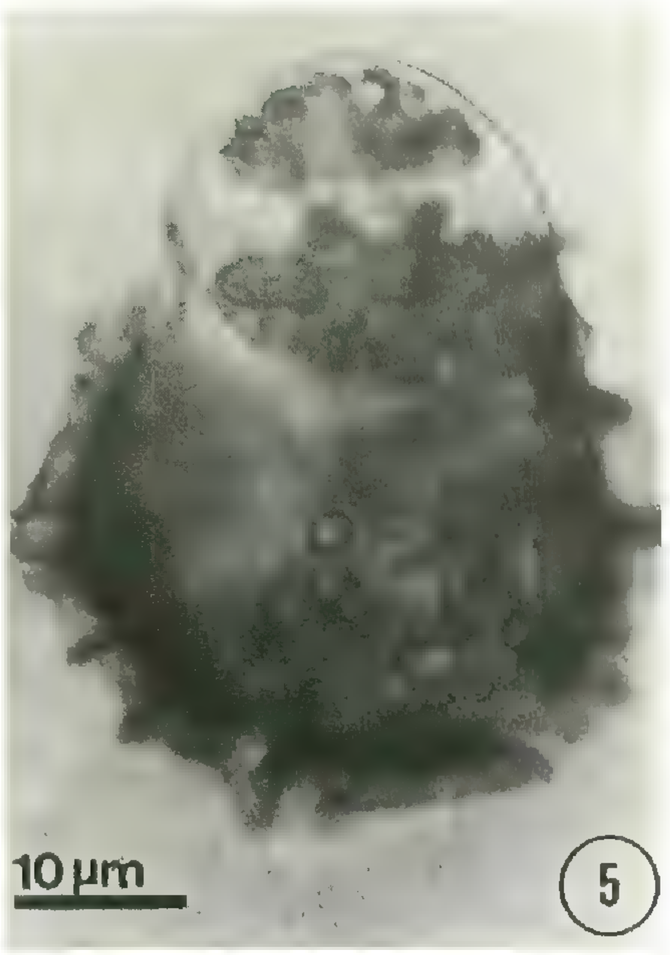


2



3

4



5

6

7

8

9

the margin of the prothallus. In our species, the adult stages show very sparse marginal hairs near the shallow notch.

There are slight variations in the shape of the vegetative thallus. In *Bolbitis portoricensis* (day 37 to 50) they are cordate-spatulate with an inconspicuous meristematic zone and weakly defined wings; others are cordate, with a slightly deep to very deep apical notch, and isodiametric wings; still others are spatulate with a shallow meristematic area and more or less isodiametric wings. All gametophytes are nearly glabrous, with very scarce marginal hairs near the shallow notch. Rhizoids are located on the central basal region (Figs. 13, 14, 17). In *B. umbrosa* (day 50) gametophytes are cordiform with poorly defined wings and meristem and, in some cases, with very scarce marginal hairs near the apex. By day 79 they are cordate with a deep pluricellular meristem and isodiametric wings (Fig. 16).

In *Bolbitis bernoullii* the adult thallus is cordate-spatulate with more or less isodiametric and thin wings, a pluricellular meristem located in a small notch, abundant rhizoids in the basal region, sparse marginal hairs by days 65 to 75, and antheridia randomly distributed over the lamina (Figs. 5, 15, 23).

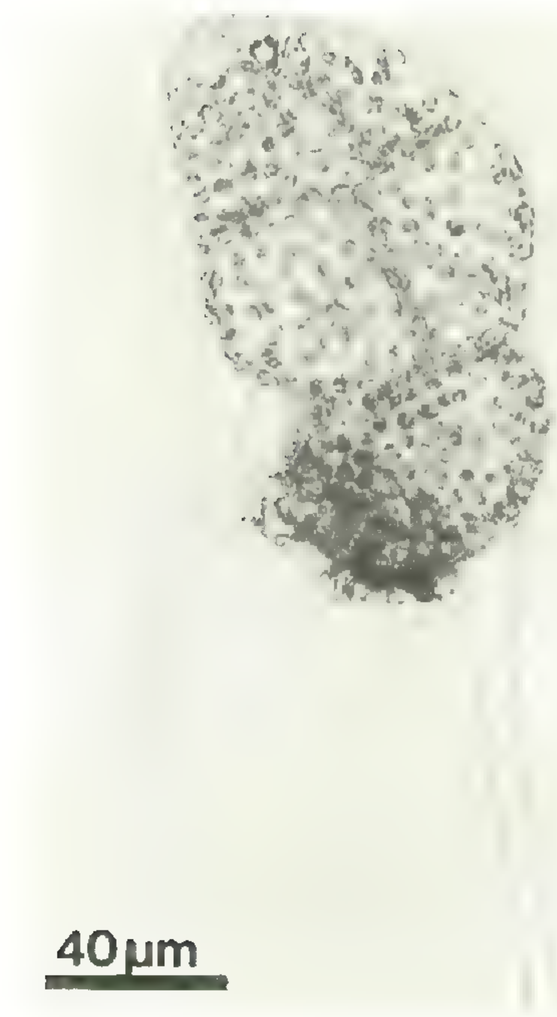
Adult gametophytes of *Bolbitis umbrosa* reach maturity between days 70 and 80. They are spatulate, with undefined wings and an apical, pluricellular meristem, have naked antheridia and abundant basal rhizoids (Fig. 24). In *B. portoricensis* there is variation in the adult phase: they can be cordiform-spatulate with isodiametric wings (day 37–62), cordiform-reniforme with wide wings and an undefined apical meristem (day 50–78), or cordiform-spatulate with short, isodiametric wings, a well defined pluricellular meristem, a dense cushion with antheridia, and smooth to slightly undulate margins (day 50 to 78) (Figs. 19, 22, 25). Laminar, bifurcate gametophytes, each bifurcation with its own apical meristem, commonly develop in cultures of *B. portoricensis* (Fig. 18).

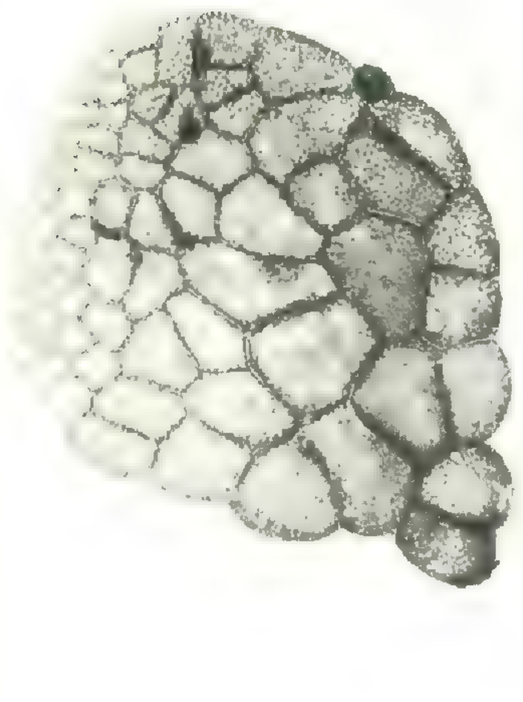
Bolbitis portoricensis and *B. umbrosa* develop a 6- to 8-celled thick, dense cushion on which the sexual organs are located. The more or less centrally located rhizoids are usually restricted to the lower surface of the cushion, and are frequently found in clusters. Rhizoids are thin and hyaline except in *B. bernoullii* where the cushion is not well defined. No collenchyma-like thickenings were seen in our species.

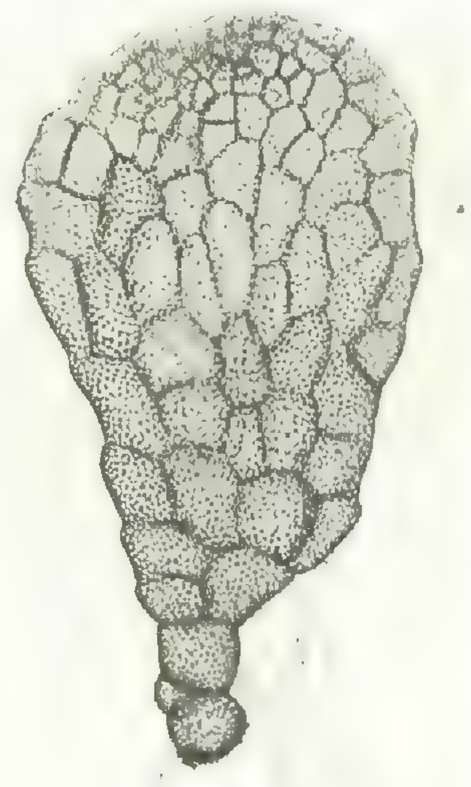
Sparse marginal hairs develop near the meristematic zone of mature thalli in *Bolbitis portoricensis* after the development of the cushion (Figs. 19–21). The hairs consist of 2 or 4 cells and vary in frecuency. Rhizoids in *B. bernoullii, B. portoricensis* and *B. umbrosa* are unicellular extensions of the superficial cells. They have brown cells walls, with thin, long and tangled chloroplasts.

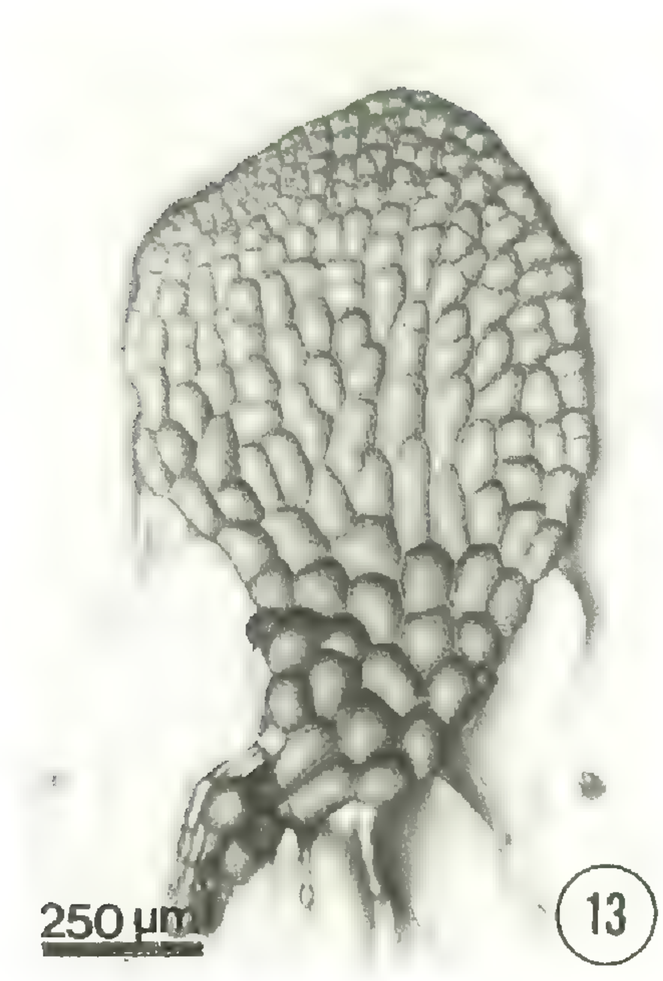
←

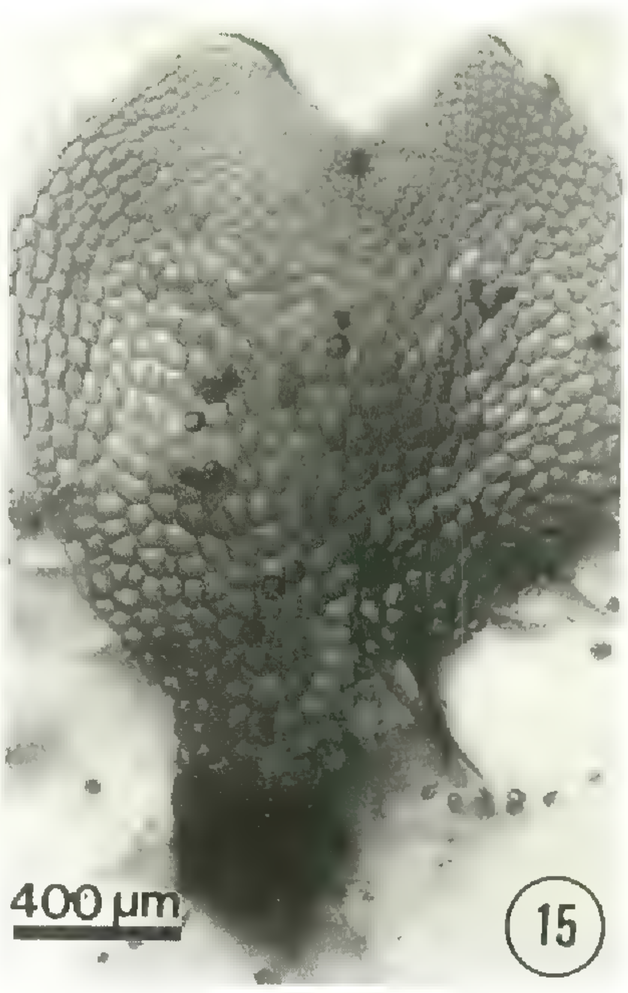
FIGS. 1–9. Spore germination and filamentous phases in *Bolbitis*. 1. Spore of *B. bernoullii*. 2. Spore of *B. portoricensis*. 3. Spore of *B. umbrosa*. 4. Germination in *B. umbrosa*, 8 days. 5. Two-celled filament in *B. bernoullii*, 20 days. 6. Three-celled filament in *B. umbrosa*, 16 days. 7. Three-celled filament in *B. portoricensis*, 9 days. 8. Six-celled filament in *B. portoricensis*, 15 days. 9. Beginning of the laminar phase in *B. portoricensis*, 16–22 days. p= perine, pc= prothallial cell, r=rhizoid.

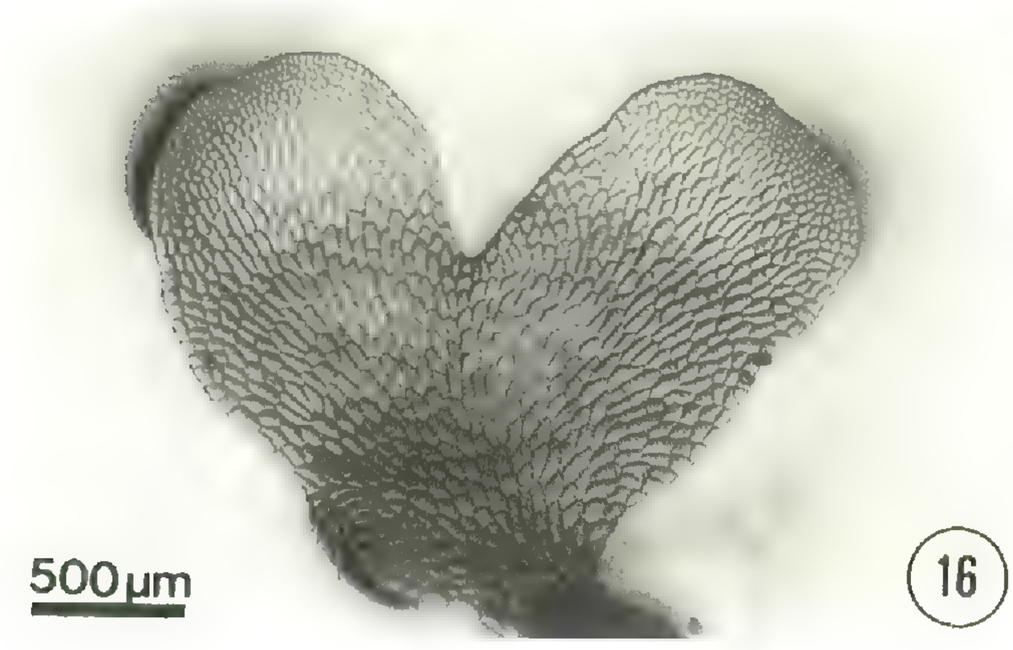


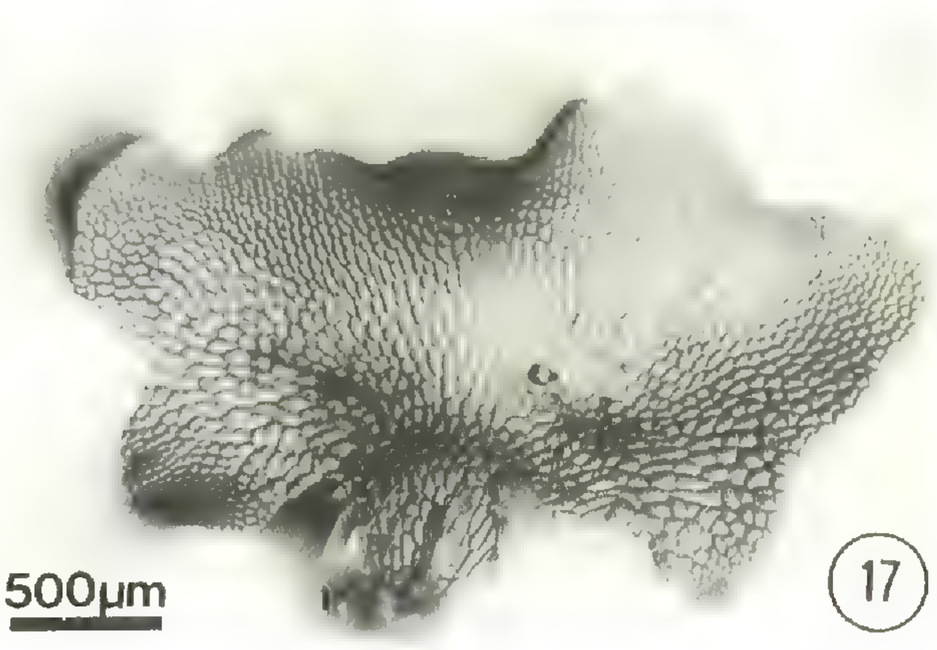












FIGS. 10–17. Young plate phases of *Bolbitis*. 10–12. Beginnings of the laminar phase in *B. umbrosa*, 16–37 days. 13–14. Laminar phases in *B. portoricensis*, 44–58 days. 15. Laminar gametophyte in *B. bernoullii*, 65–71 days. 16. Cordiform gametophyte with a deep notch in *B. umbrosa*, 50 days. 17. Gametophyte reniform with wide wings in *B. portoricensis*, 50 days.

Rhizoids develop over the ventral surface near the cushion region and are present from the earliest developmental stages.

Vegetative propagation is present in old thalli 6–8 months after sowing. In *Bolbitis portoricensis*, new gametophytes can develop from marginal cells, and by these means perpetuate the growth of the sexual generation.

The gametangia are characteristic of typical leptosporangiate ferns and the antheridia develop before the archegonia (Figs. 22–28). The antheridium is hemispherical, slightly flattened dorsi-ventrally and composed of three cells: a discoidal basal cell, a cylindrical central cell, and a lid-shaped opercular cell. These three cells surround the spermatogenous mass; during dehiscence, the opercular cells break off or collapse, liberating the antherozoids. Antheridia frequently grow among the tangled rhizoids of mature thallus. These antheridia develop superficially and are irregularly distributed over the entire lower surface, on the wings, and on the sides of the cushion. They develop in *B. bernoullii*, *B. portoricensis* and *B. umbrosa* from day 79 to 121 (Figs. 22–24, 26–28).

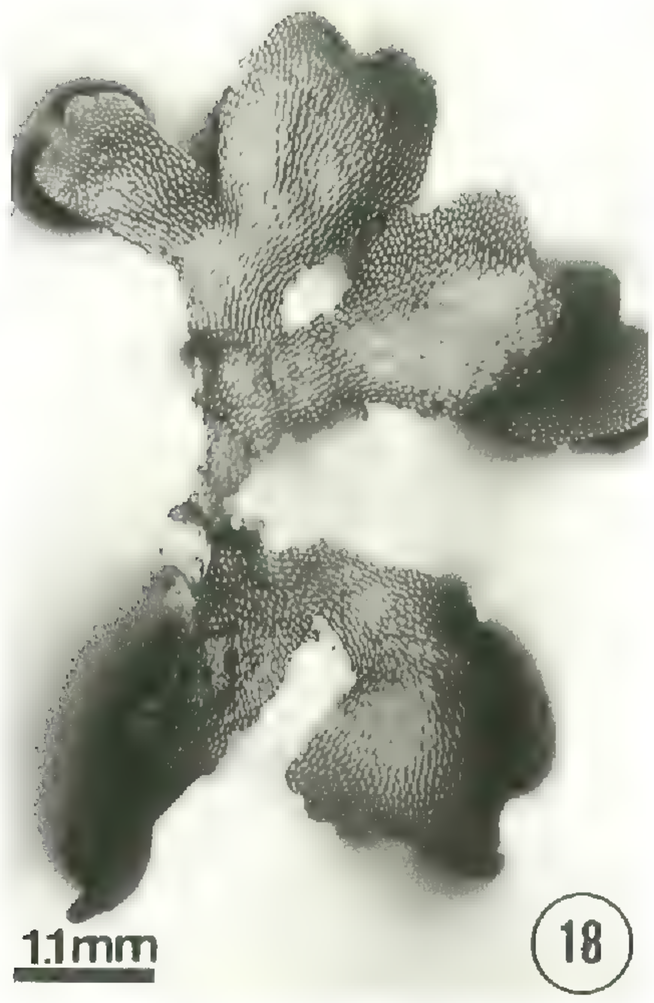
Archegonia generally develop one or two weeks after the antheridia. Archegonia are restricted to the cushion and to the area surrounding the notch. They have elongate necks that curve towards the basal region of the gametophyte. The neck canal cell is single, bi-nucleate and, near maturity, conspicuously swollen. The mouth has the four characteristic opercular cells. In *Bolbitis portoricensis* archegonia develop between day 62 and 79, in *B. bernoullii* by day 79, and in *B. umbrosa* from days 71 to 86 (Figs. 29–31).

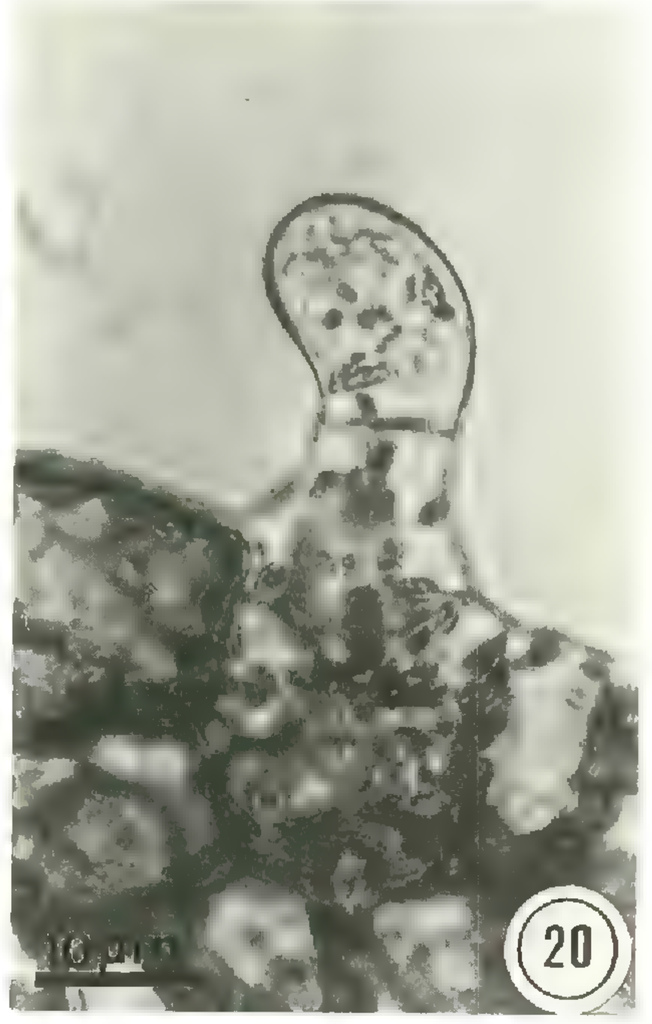
Sporophytes developed only in *Bolbitis portoricensis*, they appeared 6 to 8 months after spore germination (Fig. 32). The first juvenile leaf is shortly petiolate, simple, elongate and has a solitary or bifurcated vascular supply and a few unicellular, papillate hairs distributed along the blade. The lamina has polocytic-type stomata, wherein the guard cells are joined at one extreme (pole) and surrounded by a subsidiary cell (Fig. 33). This type of stomata is found in many leptosporangiate ferns including the Lomariopsidaceae. Their size varies from 35 to 65 μm (Van Cotthem, 1968, Hennipman, 1977). *Bolbitis portoricensis* clearly showed epidermal cells with undulated contours (see Fig. 33).

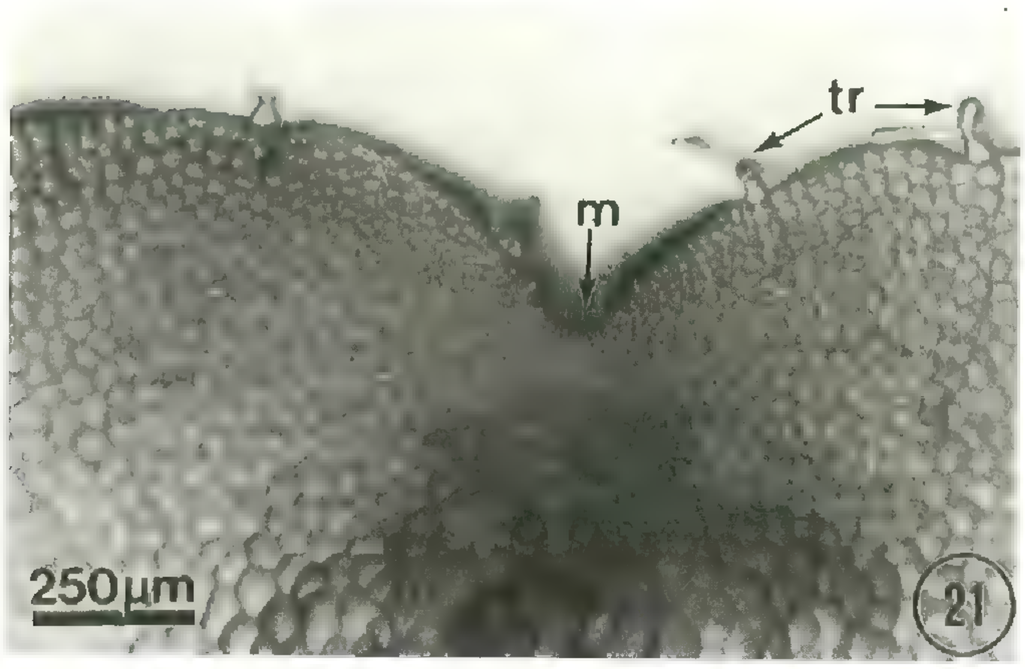
Discussion

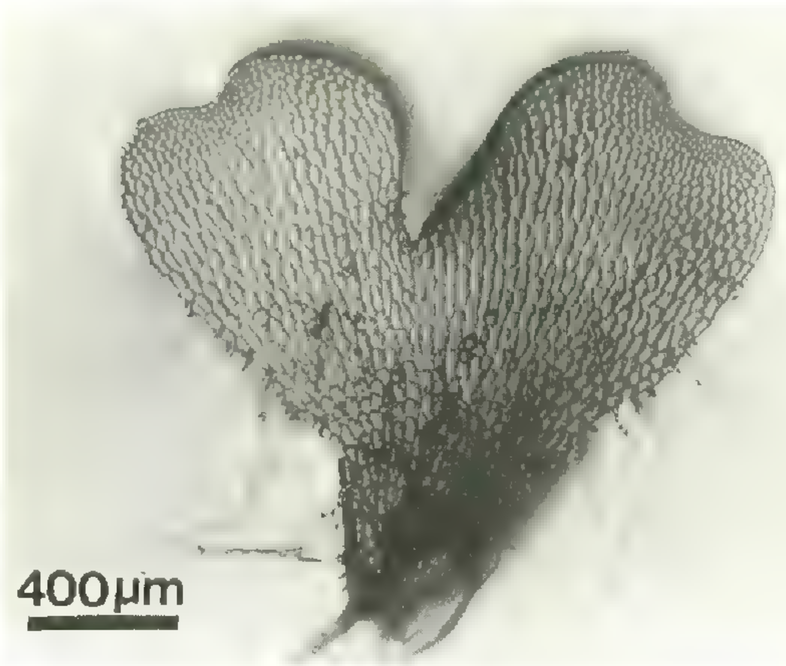
Our observations show that the development of the gametophyte in *Bolbitis bernoullii, B. portoricensis* and *B. umbrosa* is uniform. The few differences noted were insignificant and, fundamentally, all can be associated with time of development: the germination times of three species are asynchronous. Similarities found include monolete spores, *Vittaria*-type germination, *Drynaria*-type prothallial development, and gametangia of the advanced type for leptosporangiate ferns. Spore size in *Bolbitis* was found to be in agreement with data given by Hennipman (1970, 1977).

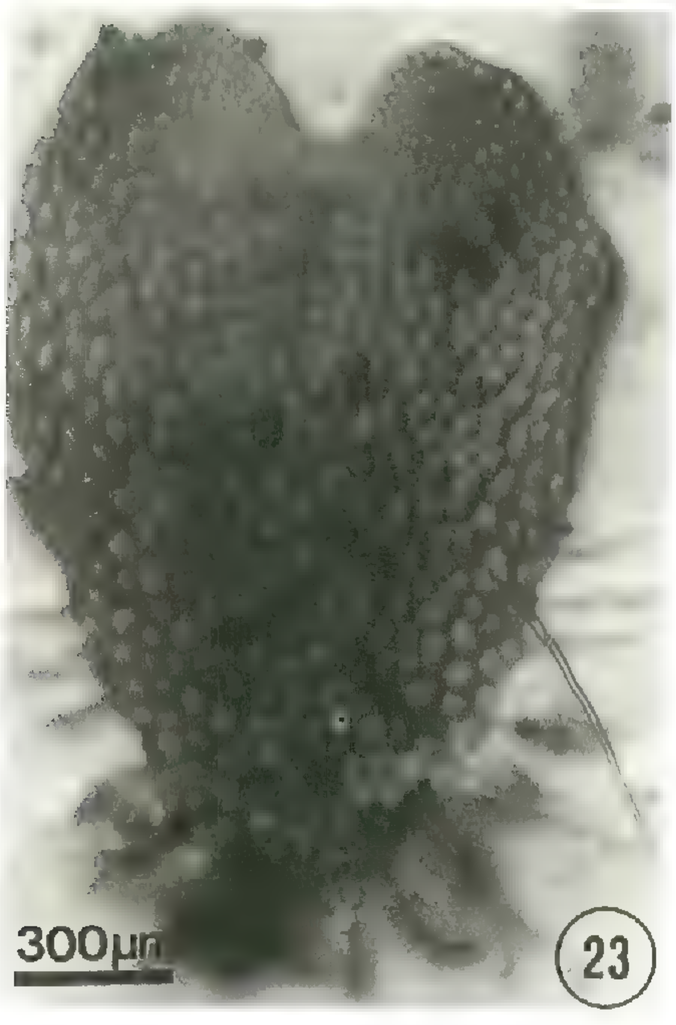
We found several differences. The spores are a little larger in *Bolbitis bernoullii* than in *B. portoricensis* and *B. umbrosa*. *Bolbitis bernoullii* has

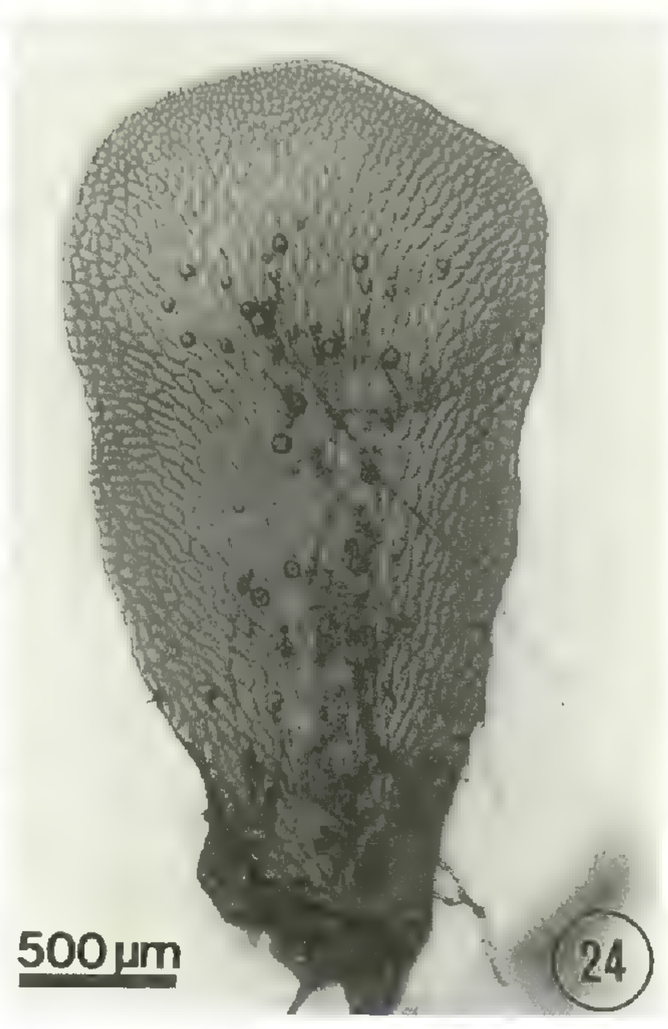


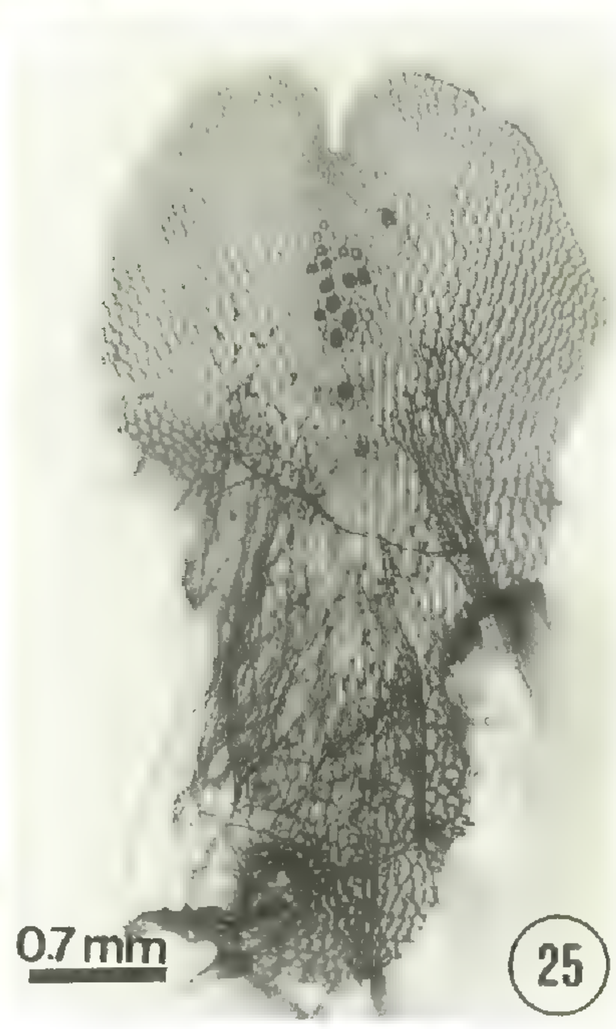












FIGS. 18–25. Adult gametophytes of *Bolbitis*. 18. Branched gametophyte in *B. portoricensis*, 90 days. 19. Cordiform-spatulate gametophyte with hairs in *B. portoricensis*, 52 days. 20. Marginal hair in *B. portoricensis*, 52 days. 21. Meristematic zone with pluricellular and marginal branched hairs in *B. portoricensis*, 107 days. 22. Gametophytes with a deep notch in *B. portoricensis*,

a wider perine than *B. portoricensis*. The germinative filaments are short, 5 to 7 cells, in *B. umbrosa*, and longer, 7 to 14 cells, in *B. bernoullii* and *B. portoricensis*. There is also variation in the adult shapes of the gametophytes. The cushion is not well defined in *B. bernoullii*, but is in the other two species. Antheridia develop between day 79 and day 121 in all three species, and the archegonia appear between days 62 and 79 in *B. portoricensis* and between days 71 and 86 in *B. bernoullii* and *B. umbrosa*.

Nayar and Kaur (1964a) reported the presence of collenchyma-like thickenings on the corners and lateral walls of cells in the gametophytes wings in *B. crispatula, B. costata* and *B. subcrenata*. Such thickenings were absent however, in the species studied here. This special type of thickening in walls of prothallial cells is characteristic of *Anemia* (Schizaeaceae; Stokey, 1951) and is uncommon in other fern genera.

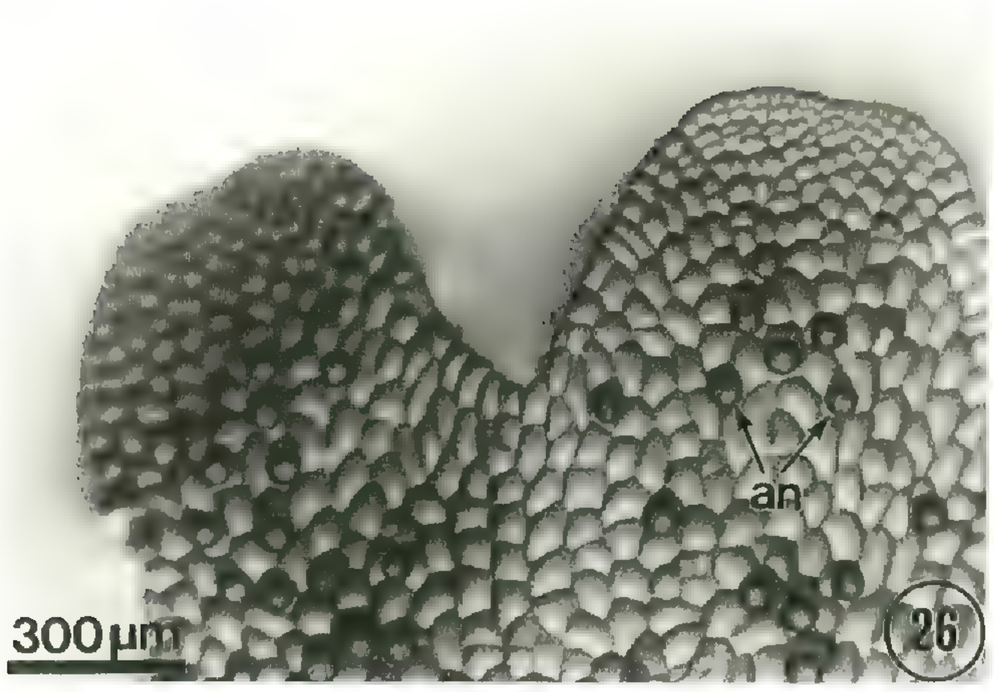
Bolbitis shares many gametophytic and sporophytic characteristics with *Egenolfia* and *Elaphoglossum* as well as other genera of the Lomariopsidaceae. These similarities include monolete spores with a prominent perine, *Vittaria*-type germination, *Drynaria*-type development, and advanced gametangia form. Differences occur in the shape of the adult gametophyte, which can be cordate or ribbon-shaped, in the presence of hairs, longevity and growth rate.

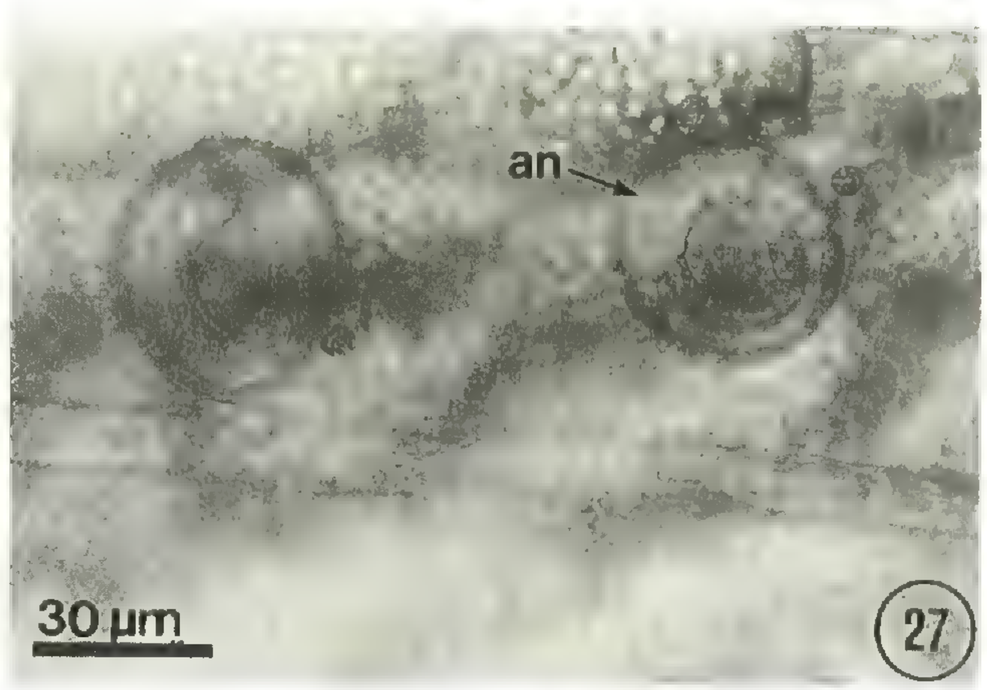
Although hairs in the gametophytic and sporophytic phases are used as taxonomic tools, our current knowledge is insufficient to assess their taxonomic value in this group (Nayar, 1956). Nayar and Kaur (1965b) statements regarding the presence of unicellular, marginal, papillate, non-glandular scattered hairs, could refer to their presence on gametophytes of *B. heteroclita*. Unicellular hairs with an extracellular waxy secretion mentioned (Nayar and Kaur, 1965b) for *B. presliana* but this needs to be confirmed. The prothallus in *Bolbitis* is naked during early phases of development. Two- to three-celled uniseriate hairs, with swollen terminal cells, are produced near the prothallial apex in *B. subcrenata* and *B. semicordata*. Each hair has a 1- to 4-cell long stalk, with a swollen, brown (from tannins) terminal cell that gives it a bulbous aspect, as *B. heteroclita* and *B. repanda* (Nayar and Kaur, 1971, Atkinson 1973). These hairs have not been seen in our species.

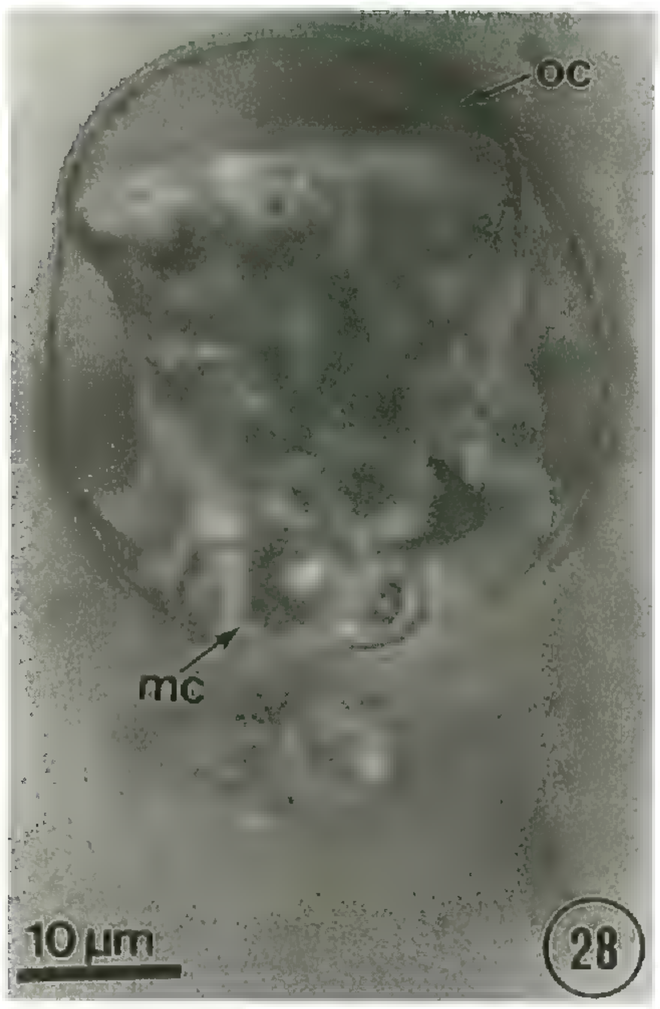
Hairs generally develop when the prothallial lamina becomes cordiform; they are present in vegetative and adult forms. We have seen sparse bicellular, short marginal hairs located along the sides of the meristematic notch, and dispersed on the wings, and the anterior part of the gametophyte in *B. bernoullii*. Such hairs are not glandular, have sparse chloroplasts and lack the swollen terminal cells as seen in *B. umbrosa* (day 50) and in *B. portoricensis* (day 62). Stokey and Atkinson (1957, 1964) mention glabrous gametophytes in *Bolbitis*.

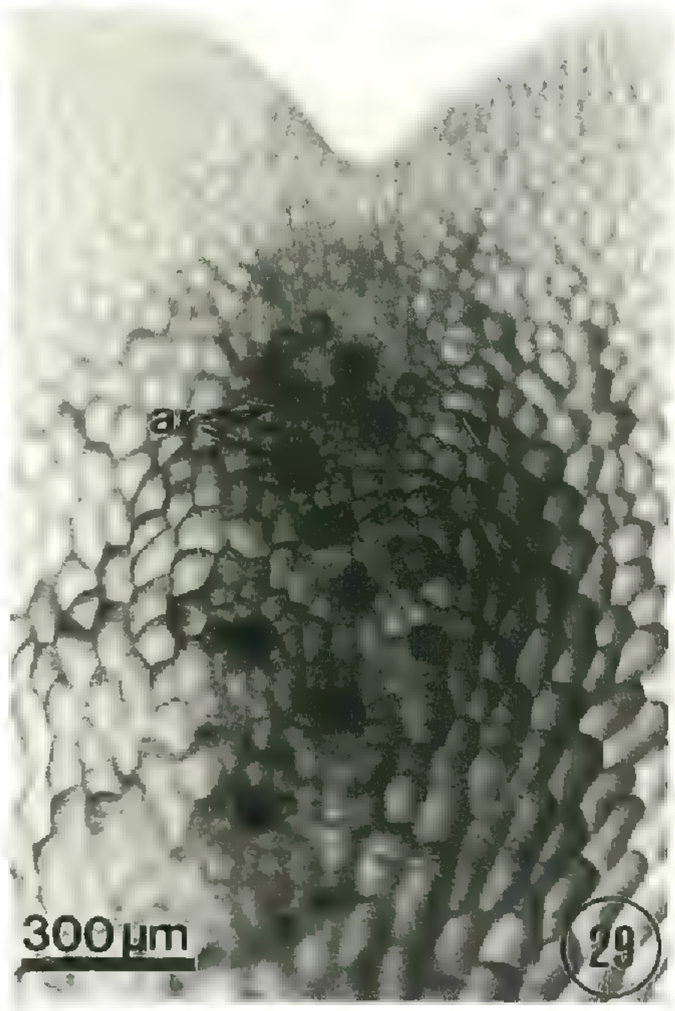
←

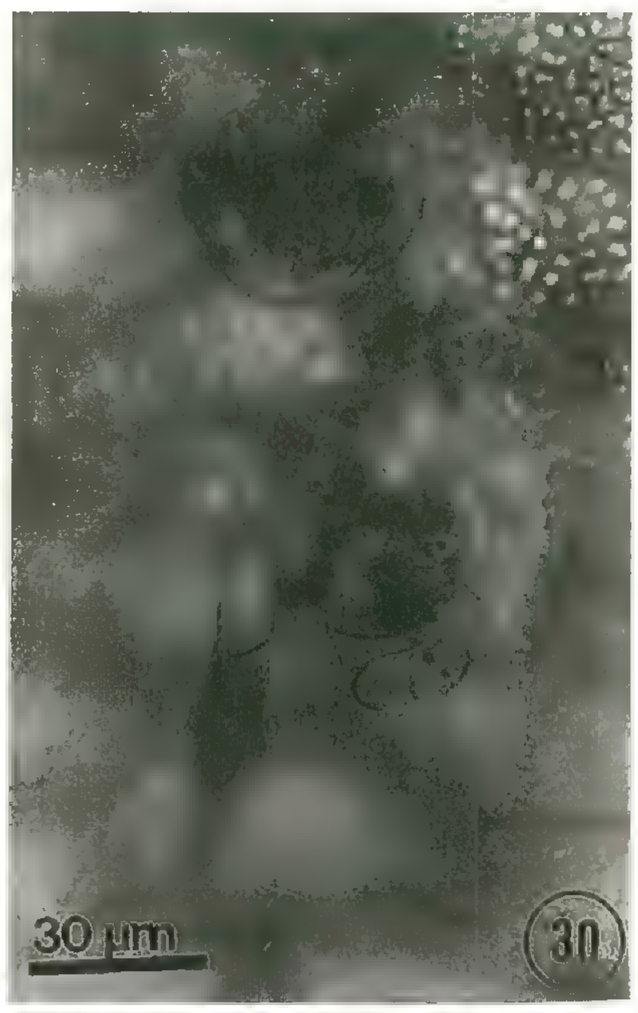
50 days. 23. Gametophyte with antheridia in *B. bernoullii*, 79 days. 24. Spatulate gametophyte with antheridia in *B. umbrosa*, 78–79 days. 25. Spatulate gametophyte with archegonia in *B. portoricensis*, 78–91 days. m= meristematic zone, tr= hair.

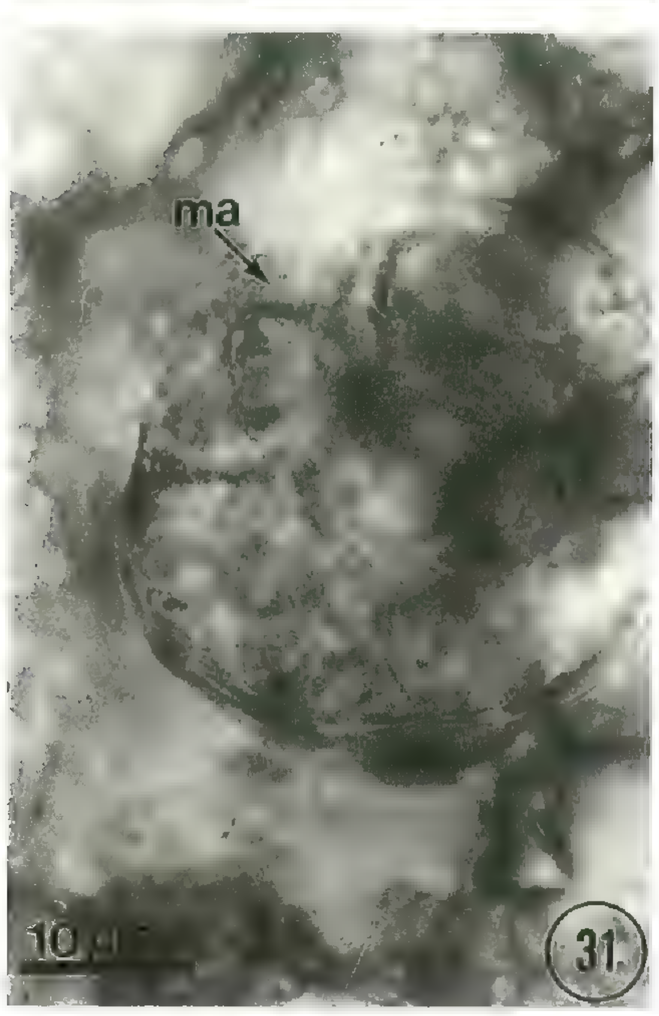


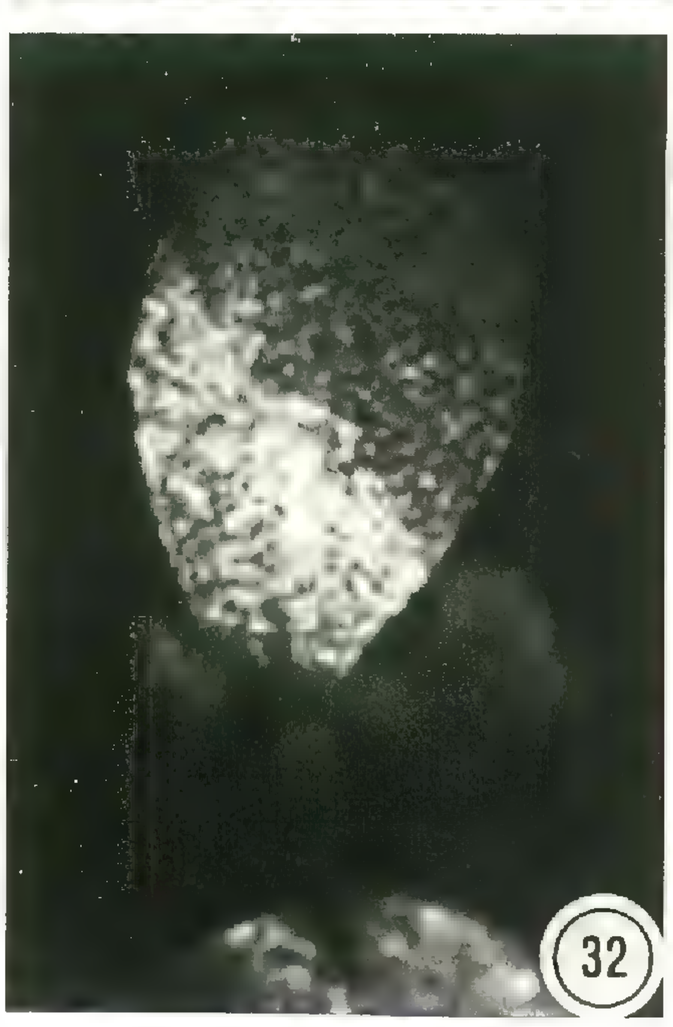












FIGS. 26–33. Gametangia and sporophytes of *Bolbitis*. 26. Antheridia on the wings in *B. portoricensis*, 79 days. 27. Antheridia in *B. portoricensis*, 91–95 days. 28. Antheridia in *B. umbrosa*, 79–86 days. 29. Archegonia on the cushion in *B. portoricensis*, 78 days. 30–31. Mouths of archegonia in *B. portoricensis*, 79–91 days. 32. First leaves of the sporophyte in *B. portoricensis*, 150 days. 33. Polocytic stomata in *B. portoricensis*, 150 days. An= antheridium, ar= archegonium, oc= opercular cell, ma= mouth of archegonium, mc= medial cell.

AMERICAN FERN JOURNAL

Volume 96

2006

QUARTERLY JOURNAL OF THE AMERICAN FERN SOCIETY

Editor

R. James Hickey
Botany Department, Miami University
Oxford, OH 45056
hickeyrj@muohio.edu

Associate Editors

Gerald J. Gastony
Department of Biology, Indiana University
Bloomington, IN 47405-6801

Gary K. Greer
Biology Department, Grand Valley State University
Allendale, MI 49401

Christopher H. Haufler
Department of Ecology and Evolutionary Biology, University of Kansas
Lawrence, KS 66045-2106

Robbin C. Moran
New York Botanical Garden
Bronx, NY 10458-5126

James H. Peck
Department of Biology University of Arkansas–Little Rock
Little Rock, AR 72204

Table of Contents for Volume 96
(A list of articles arranged alphabetically by author)

AULD, J. R. (see J. E. WATKINS, JR.) 83

BARCELONA, J. F., N. E. DOLOTINA, G. S. MADROÑERO, W. G. GRANERT and D. D. SOPOT. The Ferns and Fern Allies of the Karst Forests of Bohol Island, Philippines 1

BICKSLER, A. J. (see J. E. WATKINS, JR.) 83

BRADLEY, K. A. *Thelypteris sancta* (L.) Ching, New for Florida and the Continental United States 112

CHANG, H.-M. (see W. SINOHARA) 96

CONANT, D. S. (see P. KORALL) 45

DOLOTINA, N. E. (see J. F. BARCELONA) 1

GIUDICE, G. E. (see E. R. DE LA SOTA) 75

GRANERT, W. G. (see J. F. BARCELONA) 1

GUO, X.-S. and B. LI. A New Species of *Microlepia* (Dennstaedtiaceeae) from Mt. Micangshan, China 80

HICKEY, R. J. Review – Ferns of Northeastern and Central North America, 2nd ed. 100

HSU, T.-W. W. (see W. SINOHARA) 96

IMPERATO, F. Kaempferol 3-*O*-(acetylrutinoside), A New Flavonoid and Two New Fern Constituents, Quercitin 3-*O*-(acetylglucoside) and 3-*O*-(acetylrutinoside) from *Dryotperis villarii* 93

IMPERATO, F. The New Flavone Ester Apigenin 7-*O*-*p*-hydroxybenzoate and Three Di-*C*-Glycosylflavones from *Pteris vittata* 62

ITAM, K. (see U. YUSUF) 134

JACOB, N. M. (see U. YUSUF) 134

KAISER, K. (see J. E. WATKINS, JR.) 83

KAWAHARA, A. Y. (see J. E. WATKINS, JR.) 83

KESSLER, M. AND A. R. SMITH. *Megalastrum* (Dryopteridaceae – Pteridophyta) in Bolivia, with Descriptions of Six New Species 31

KORALL, P., D. S. CONANT, H. SCHNEIDER, K UEDA, H. NISHIDA and K. M. PRYER. On the Phylogenetic Position of *Cystodium*: It's Not a Tree Fern – It's a Polypod! 45

LEICHT, S. A. (see J. E. WATKINS, JR.) 83

LI, B. (see X.-S. GUO) 80

Liu, H., Q. F. Wang and W. C. Taylor. Morphological and Anatomical Variation in Sporophylls of *Isoetes sinensis* Palmer (Isoetaceae), an Endangered Quillwort in China 67

Luna, M. L. (see E. R. de la Sota) 75

Madroñero, G. S. (see J. F. Barcelona) 1

Mehltreter, Klaus. Leaf Phenology of the Climbing Fern *Lygodium venustum* in a Semideciduous Lowland Forest on the Gulf of Mexico 21

Mendoza-Ruiz, A. (see B. Pérez-García) 115

Moore, S.-J. (see W. Sinohara) 96

Moran, R. C. Review – Flora de la República de Cuba: Series A. Plantas Vasculares. Fascículo 11 66

Murakami, N. (see W. Sinohara) 96

Nester-Hudson J. E. (see T. E. Willis) 127

Nishida, H. (see P. Korall) 45

Pérez-García, B. and A. Mendoza-Ruiz. Gametophyte Morphology in Three Mexican Species of *Bolbitis* (Lomariopsidaceae) 115

Pryer, K. M. (see P. Korall) 45

Schmitt, J. L. and P. G. Windisch. Growth Rates and Age Estimates of *Alsophila setosa* Kaulf. in Southern Brazil 103

Schneider, H. (see P. Korall) 45

Sinohara, W., T.-W. Hsu, S.-J. Moore, H.-M. Chang and N. Murakami. *Deparia longipes* (Woodsiaceae) Native to Taiwan 96

Smith, A. R. (see M. Kessler) 31

Sopot, D. D. (see J. F. Barcelona) 1

Sota, de la, E. R., G. E. Giudice and M. L. Luna. *Blechnum ludificans* Herter, An Overlooked Fern from South America 75

Sukari, M. A. (see U. Yusuf) 134

Taylor, W. C. (see H. Liu) 67

Ueda, K. (see P. Korall) 45

Wang, Q. F. (see H. Liu) 67

Watkins, J. E., A. Y. Kawahara, S. A. Leicht, J. R. Auld, A. J. Bicksler and K. Kaiser. Fern Laminar Scales Protect Against Photoinhibition from Excess Light 83

Whittier, D. P. Gametophytes of Four Tropical, Terrestrial *Huperzia* Species (Lycopodiaceae) 54

Willis, T. E. and J. E. Nester-Hudson. Characterization Of A *Thelypteris* Hybrid From Walker County, Texas 127

WINDISCH, P. G. (see J. L. SCHMITT) 103

YUSUF, U., N. M. JACOB, M. A. SUKARI, and K. ITAM. Chemotaxonomic Survey of Flavonoids from *Sphaerostephanos* (Thelypteridaceae) of Peninsular Malaysia 134

Volume 96, number 1, January–March, pages 1–44, issued 4 May 2006

Volume 96, number 2, April–June, pages 45–66, issued 7 September 2006

Volume 96, number 3, July–September, pages 67–102, issued 15 December 2006

Volume 96, number 4, October–December, pages 103–144, issued 7 May 2007

A young sporophyte developed after 6–8 months in the cultivation experiments only in *B. portoricensis*, and vegetative propagation took place.

Most of our observations agree with those of Nayar and Kaur (1964a, 1965a), and Atkinson (1973). Our findings agree with Hennipman's (1977) observations that branched hairs are absent. This is in disagreement with statements by Nayar and Kaur (1964a, 1965a) that they are present. Such hairs are otherwise unknown for the Lomariopsidaceae.

Stokey (1951) and Nayar & Kaur (1971) suggested that useful information on gametophyte morphology might be found in spore germination patterns, manner of development of the cell plate and the meristemastic regions, form of the adult prothallus, type, position and time of appearence of hairs when present, and reproductive structures, especially the antheridium. Consistent comparative data from careful observations of these gametophytic features have been used, in further elucidating the phylogenetic relations of pteridophytes. The results of our comparative studies of the sexual phase of *Bolbitis* contribute to more detailed information of gametophyte morphology of leptosporangiate ferns, which is useful in understanding the reproductive biology the genus.

Acknowledgments

The authors wish to express their gratitude to the anonymous reviewers for their suggestions and criticisms of the manuscript. R. James Hickey also read the manuscript and offered suggestions. We thank J. Lodigiani for photographic assistance.

Literature Cited

Atkinson, L. M. 1973. The gametophyte and family relationships. in A. C. Jermy, J. A. Crabbe and B. A. Thomas, eds. *The phylogeny and classification of the ferns*. J. Linn. Soc. Bot. 67:73–90.

Hennipman, E. 1970. Electron and light microscopical observations of the perine of the spores of some *Bolbitis* (Filices). Acta Bot. Neerl. 9:671–680.

Hennipman, E. 1977. A monograph of the fern genus *Bolbitis* (Lomariopsidaceae). Leiden Botanical Series No. 2:1–331.

Mendoza-Ruiz, A. 2001. *Morfogénesis de la fase sexual de pteridofitas mexicanas, familia Dryopteridaceae*. Tesis Maestría, UNAM.

Mendoza-Ruiz, A. and B. Pérez-García. 2003. Comparative analysis of the sexual phase of *Phanerophlebia* (Dryopteridaceae) in Mexico. Can. J. Bot. 81:501–516.

Mickel, J. T. and A. R. Smith. 2004. *The pteridophytes of Mexico*. The New York Bot. Gard. Press, Bronx, New York.

Moran, R. C. 1995. Lomariopsidaceae. Pp. 247–250, in R. C. Moran and R. Riba, eds. *Flora Mesoamericana*, 1: *Psilotaceae a Salviniaceae*. Instituto de Biología, Universidad Nacional Autónoma de México, Missouri Botanical Garden y The Natural History Museum (London), México, D. F.

Nayar, B. K. 1956. Correlation between gametophytic and sporophytic trichomes in ferns. Sci. & Cult. 21:455–457.

Nayar, B. K. 1960. Morphology of two Indian Species of *Bolbitis*. J. Indian Bot. Soc. 39:259–277.

Nayar, B. K. and S. Kaur. 1964a. Ferns of India-XI: *Bolbitis*. Bull. Nat. Bot. Gard. Lucknow 88:1–74.

Nayar, B. K. and S. Kaur. 1964b. Ferns of India-XIII: *Egenolfia*. Bull. Nat. Bot. Gards. Lucknow 100:1–38.

NAYAR, B. K. and S. KAUR. 1965a. Studies on the fern genera *Bolbitis* and *Egenolfia*. I. The adult sporophyte of some species. J. Linn. Soc. Bot. 59:127–140.

NAYAR, B. K. and S. KAUR. 1965b. Studies on the fern genera *Bolbitis* and *Egenolfia* II: The gametophytes and the juvenile sporophytes. J. Linn. Soc., Bot. 59:141–154.

NAYAR, B. K. and S. KAUR. 1965c. Spore morphology of the Lomariopsidaceae. J. Palynol. 1:10–26.

NAYAR, B. K. and S. KAUR. 1969. Types of prothallial development in homosporous ferns. Phytomorphology 19:179–188.

NAYAR, B. K. and S. KAUR. 1971. Gametophytes of homosporous ferns. Bot. Rev. 37:295–396.

STOKEY, A. G. 1951. The contribution by the gametophyte to classification of homosporous ferns. Phytomorphology 1:39–58.

STOKEY, A. G. and L. R. ATKINSON. 1957. The gametophyte of some American species of *Elaphoglossum* and *Rhipidopteris*. Phytomorphology 7:275–292.

STOKEY, A. G. and L. R. ATKINSON. 1964. Comparative morphology of the gametophyte of homosporous ferns. Phytomorphology 14:51–70.

TRYON, A. F. and B. LUGARDON. 1991. *Spores of the Pteridophyta: surface, wall structure, and diversity based on electron microscope study*. Springer-Verlag, New York.

TRYON, R. M. and A. F. TRYON. 1982. *Ferns and allied plants with special reference to tropical America*. Springer-Verlag, New York.

VAN COTTHEM, W. R. J. 1968. *Vergelijkend-morfologische studie van de stomata bij de Filicopsida*. Doct. Proefschrift, Rijksuniversiteit Gent, Gent, Belgium.

American Fern Journal 96(4):127–133 (2006)

Characterization of a *Thelypteris* Hybrid from Walker County, Texas

TRACY E. WILLIS and JOAN E. NESTER-HUDSON
Department of Biological Sciences, Sam Houston State University, Huntsville, TX 77341

ABSTRACT.—A putative hybrid of unknown parentage was discovered in Walker County, Texas. Taxonomic features resemble both *Thelypteris kunthii* and *Thelypteris ovata* var. *lindheimeri* indicating hybridity. Spores of the putative hybrid examined by microscopy appeared malformed and were sterile with 0% germination. Guard cell measurements were intermediate to the presumed diploid and tetraploid parental species suggesting that the hybrid is triploid. Qualitative and quantitative phenetic characters examined were an array of parental and intermediate characters, suggesting that it had resulted from a cross between *Thelypteris kunthii* and *Thelypteris ovata* var. *lindheimeri*.

Ferns have extensive hybridization (Wagner, 1969) and Knobloch (1976) listed 620 fern hybrids, including some alloploids of known parentage. Fertile and sterile hybrids of *Thelypteris* have been described, primarily from Florida and Mexico (Mickel *et al.*, 1966). Smith (1993) described hybrids of *Thelypteris* in Florida between *Thelypteris kunthii* (Desv.) C. V. Morton and *Thelypteris ovata* R. P. St. John var. *ovata*, and between *T. kunthii* and *Thelypteris augescens* (Link) Munz *et* I. M. Johnson.

A *Thelypteris* was discovered on the grounds of the Sam Houston Memorial Museum (SHMM) in Walker County, Texas. The parentage of this taxon is not known. Alan R. Smith (personal communication) suggested it was a hybrid, and that the parents could be *Thelypteris kunthii* and *Thelypteris ovata* var. *lindheimeri* (C. Christensen). Native ranges of the proposed parents do not overlap (Smith, 1993). *Thelypteris kunthii* is native to the southeastern U.S., with its range extending into east Texas and Walker County. *Thelypteris ovata* var. *lindheimeri* is native to north central Texas and west into the Edwards Plateau and Trans-Pecos areas (Smith, 1993; Diggs *et al.*, 1999). Although *T. ovata* var. *lindheimeri* is not native to Walker County, it is grown as an ornamental within three city blocks from the apparent hybrid on the Sam Houston State University (SHSU) campus. The goal of this study was to characterize and determine parentage of the suspected hybrid by comparing phenetic traits with *T. kunthii* and *T. ovata* var. *lindheimeri*.

MATERIALS AND METHODS

All spores, croziers, and fully developed fronds were collected in Walker County, Texas, from May 1999 until August 2001. Putative hybrid material was collected on the grounds of the SHMM. *Thelypteris ovata* var. *lindheimeri* was collected from a cultivated population on campus at SHSU. *Thelypteris kunthii* was collected from a wild population growing at the SHSU Center for

Biological Field Studies, within ten miles from campus. All spores were stored at 4°C. Herbarium specimens from all three ferns were deposited in the SHSU S. R. Warner Herbarium.

Walter M. Woodward, Curator of Collections, and Dr. Patrick Nolan, Director, of the SHMM were interviewed for a historical background of the hybrid location. Manuscripts written by Martinus H. Stougaard, the first Landscape Architect of the SHMM were consulted at the Peabody Library, SHSU. Grounds keeping and maintenance personnel were interviewed for historical records that would indicate the date *T. ovata* var. *lindheimeri* was planted on campus as an ornamental. The area surrounding the SHMM, two city blocks north, east, south, and west, was surveyed for *Thelypteris* species. Specimens were collected and examined morphologically.

Traits distinguishing the putative parents (Smith, 1971, 1993) were examined and compared with those of the hybrid. Observations and measurements were made from nine specimens of *T. ovata* var. *lindheimeri*, seven specimens of the putative hybrid, and eleven specimens of *T. kunthii*.

We assessed the following morphological characters for evidence of intermediacy in the putative hybrid: 1) overall leaf shape including blade apex; 2) presence or absence of scales on the rachis; 3) pinnae shape and attachment of middle pinnae to the rachis; 4) location of the sori; and 5) presence or absence of stalked sporangial glands. Also, spores were attached to SEM stubs with double sided tape, sputter coated to 200 Å for two minutes with gold palladium, examined, and photographed under a Scanning Electron Microscope (JEOL model JSM 6400 Scanning Microscope) at the Texas A&M University Microscopy Center, College Station, Texas. Spores were mounted in Hoyer's medium on glass slides for measurements of spore length that included the perispore. Photomicrographs were obtained after several weeks to insure maximum swelling had occurred in the spores (Barrington *et al.*, 1986),

We examined light and SEM micrographs of spores for signs of spore malformation. To test spore viability, spores from each fern were surface-sterilized and sown (Nester and Schedlbauer, 1981) under aseptic conditions (Schedlbauer, 1976) onto 1% agar solidified plates containing Parker's macroelements and Thompson's microelements (Klekowski, 1969). Germinated and ungerminated spores in three fields of view per plate were counted. We observed at least 200 spores from each of the three ferns and obtained germination percentages.

Ploidy of the hybrid was determined by statistically comparing the guard cell length of the putative hybrid and the presumed parents. Statistical analyses were performed with Minitab Student Release 12 (Minitab Inc., 1998). Analysis of variance (ANOVA) was used to determine significant differences between the putative parents and the hybrid, and results were considered statistically significant at $\alpha=0.05$. We obtained guard cell lengths from the pinnatifid apex of the leaf, where there were no sori or major veins to interfere with measurements. One to two millimeter leaf pieces were rinsed in 100% ethanol and mounted in Hoyer's medium onto glass slides. Guard cell length was measured using an ocular micrometer and photographed.

Results

Information provided by local historians indicates the putative hybrid most likely appeared near the SHMM some time in the 1930's. Martinus H. Stougaard was employed at the SHMM from 1928 to 1936 as a landscape architect. Manuscripts written by Stougaard do not indicate his participation in the synthesis of this specimen. Unfortunately, we found no direct evidence for synthetic or naturally occurring hybridization of *T. kunthii* and *T. ovata* var. *lindheimeri*. Historical records were not available to validate when *T. ovata* var. *lindheimeri* was planted as an ornamental on campus.

In the past, people in this area could have used *Thelypteris* as an ornamental. Therefore, a two city block radius surrounding the SHMM was surveyed for *Thelypteris*. *Thelypteris* was found growing in six locations. The presence of stalked sporangial glands, sori location, and guard cell size indicated three of the specimens were *T. kunthii*. The presence of stalked sporangial glands, dark hairy scales on the rachis, elongate basal segments of the middle pinnae that are parallel to the rachis, and guard cell size suggested one specimen was the hybrid in question. Persistent tan glabrous scales, middle pinnae with basal segments that are elongate and parallel to the rachis, and guard cell size indicated two specimens were *T. ovata* var. *lindheimeri*.

Characters of the three *Thelypteris* taxa are described below and shown in Table 1. Blade shape for *T. ovata* var. *lindheimeri* was ovate-lanceate. Blade shape varied from ovate-lanceate, lanceolate, to triangular for the hybrid, and was lanceolate, lanceate, to triangular in *T. kunthii*. Both *T. ovata* var. *lindheimeri* and the hybrid had a gradually to somewhat abruptly tapered apex while *T. kunthii* had a gradually tapered apex.

The basal segments of the middle pinnae were elongate and parallel to the rachis in both *T. ovata* var. *lindheimeri* and the hybrid, but not elongate and somewhat oblique to the rachis in *T. kunthii*. Pinna segments in *T. ovata* var. *lindheimeri* were oblique and curved with submarginal sori. Pinna segments in the hybrid were oblique to oblong with rounded to acute apices and supramedial to medial sori. Pinna segments were oblong with rounded to acute apices with supramedial to medial sori in *T. kunthii*.

Scales were dense on the rachis of *T. ovata* var. *lindheimeri*, sparse to dense on the rachis of the hybrid, and sparse on the rachis of *T. kunthii*. Scales on *T. ovata* var. *lindheimeri* were tannish brown, and glabrous to minutely pubescent. Scales of the hybrid and *T. kunthii* were dark brown and pubescent. Yellowish-stalked sporangial glands were present in *T. kunthii* and the hybrid, and absent in *T. ovata* var. *lindheimeri*.

SEM photographs were used to compare spore ornamentation and verify any differences and/or similarities between the hybrid and the proposed parents (Fig. 1). Spores of *T. ovata* var. *lindheimeri* were cristate with continuous wide flat crests, sparsely verrucose, with small pits (Fig. 1a). Spores of *T. kunthii* (Fig. 1b) were cristate with discontinuous thin crests, verrucose, with small pits. The hybrid spores (Fig. 1c) appeared to be cristate, with thin continuous crests, verrucose to tuberculate. Micrographs reveal hybrid spores collapsed

TABLE 1. Morphological characteristics of *T. ovata* var. *lindheimeri*, putative hybrid, and *T. kunthii*.

Character	*T. ovata* var. *lindheimeri*	putative hybrid	*T. kunthii*
Blade shape	Ovate-lanceate	Lanceolate, triangular or ovate-lanceate	Lanceolate, lanceate, or triangular
Apex	Gradually-somewhat abruptly tapered	Gradually-somewhat abruptly tapered	Gradually tapered
Basal pair middle pinnae	Elongate and parallel to rachis	Elongate and parallel to rachis	Not elongate, somewhat oblique to the rachis
Pinnae segments	Oblique to curved	Oblong, oblique to Curved	Oblong
Pinnae apex	Acute	Rounded to acute	Rounded to acute
Sorus location	Submarginal	Supramedial-medial	Supramedial-medial
Rachis Scales	Dense	Sparse-dense	Sparse
Rachis Color	Tannish brown	Dark brown	Dark brown
Rachis hair	Minutely pubescent	Pubescent	Pubescent
Sporangial Glands	Absent	Present	Present
Guard cell Length	32 ± 5 µm	36 ± 4 µm	40 ± 4 µm
Spore length (including perispore)	50 ± 4 µm	46 + 6 µm	52 ± 7 µm

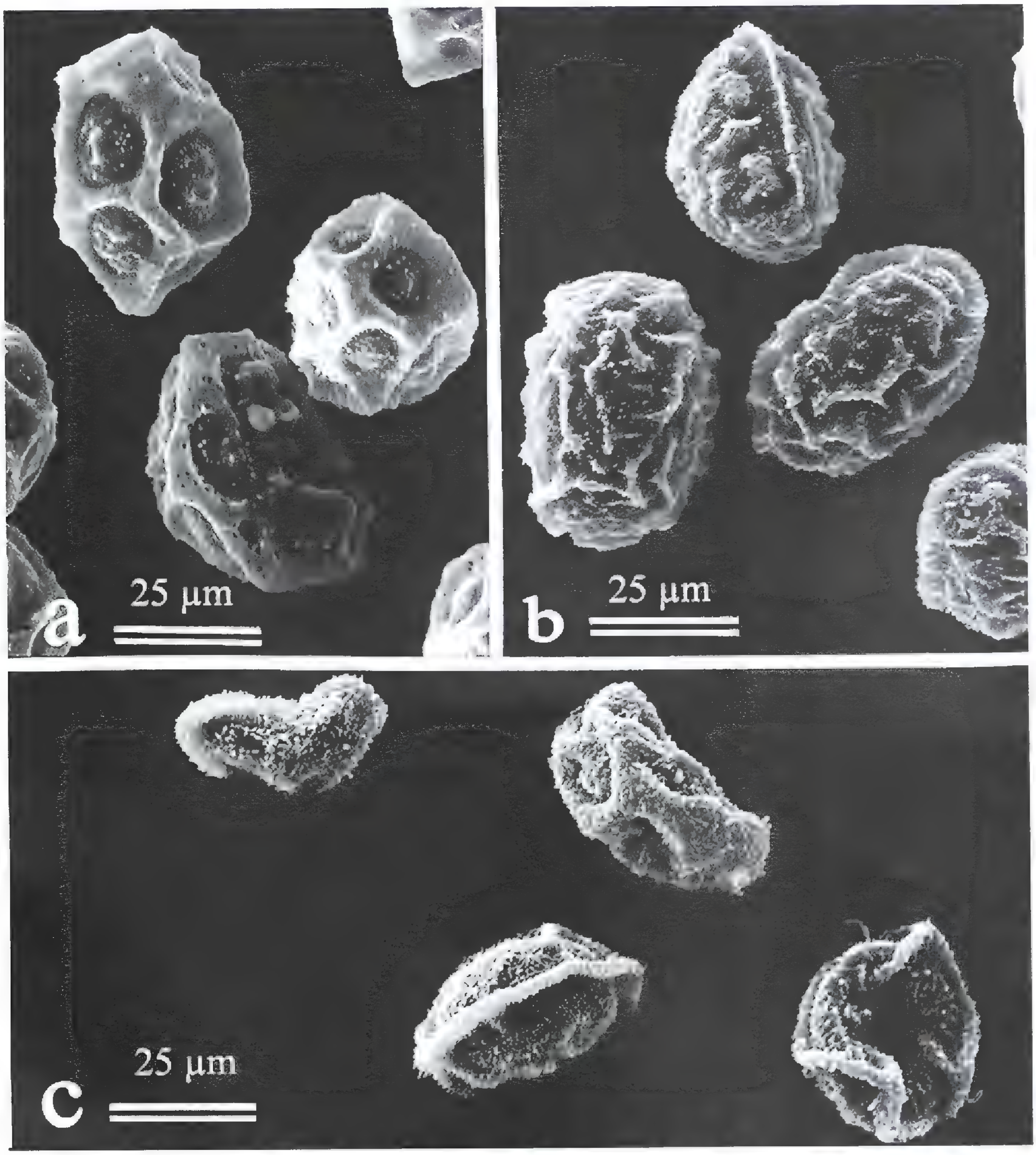


FIG. 1. SEM of spores of *Thelypteris* species and putative hybrid. *Thelypteris ovata* var. *lindheimeri* spores (a) with continuous wide flat crests and small pits, *Thelypteris kunthii* spores (b) with discontinuous thin crests, verrucose with small pits, and hybrid spores (c) with thin continuous crests, verrucose to tuberculate.

and twisted, showing concave walls and strong curved outlines, which are all characteristics of nonviable spores in hybrids. By day 18, germination rate was 65% for *T. kunthii*, 55% for *T. ovata* var. *lindheimeri*, and 0% for the hybrid.

Guard cell length for all three ferns was measured and compared for indications of ploidy level of the hybrid (Table 1). Average guard cell length

was 32 ± 5 μm for *T. ovata* var. *lindheimeri*, 36 ± 4 μm for the putative hybrid, and 40 ± 4 μm for *T. kunthii*. ANOVA showed that the three taxa were significantly different ($P<0.001$).

Spore length (including the perispore) for all three ferns was measured and compared for indications of ploidal level of the hybrid (Table 1). *T. ovata* var. *lindheimeri* had an average spore length of 50 ± 4 μm, the hybrid 46 ± 6 μm, and 52 ± 7 μm for *T. kunthii*.

Discussion

Morphological characters suggest the putative *Thelypteris* hybrid arose from a cross between *T. kunthii* and *T. ovata* var. *lindheimeri*. The place, time and circumstances of this origin are uncertain.

Thelypteris was found in six locations inside the area surveyed. From a comparison of taxonomic characters and guard cell size, we conclude that one of the six specimens collected was a hybrid, three were *T. kunthii*, and the remaining two were *T. ovata* var. *lindheimeri*. *Thelypteris* species appear to be commonly used as ornamentals in the area surveyed and are easily propagated by rhizome fragmentation.

All examined morphological characteristics of the hybrid show a combination of the features of both presumed parents. A common misconception is that hybrids are typically morphologically intermediate between their parents (Reiseberg, 1995). Reiseberg and Ellstrand (1993) found that hybrids are a mosaic of parental, intermediate, and extreme characters. Hybrids commonly express morphological intermediacy, but the characters that are governed by just one or a few genes can have parental, novel, or extreme character states (Reiseberg, 1995).

All viable spores of *T. kunthii and T. ovata* var. *lindheimeri* had germinated by day 18 of the viability test. Spores of the hybrid are malformed, a characteristic of spores in other hybrid pteridophytes (Smith, 1971; Wagner *et al.*, 1986; Brunton and Taylor, 1990; Hoshizaki, 2001), and had 0% germination, which is typical of fern hybrids with uneven chromosome numbers. Malformed spores and their nonviability are strong evidence of hybridization in homosporous pteridophytes. Although spore malformation may sometimes be due to factors other than hybridity, this combination of circumstances has occurred so commonly in ferns that the likelihood of any other explanation is exceedingly small (Wagner et al. 1986).

Cell size has been found to correlate with ploidal level within some fern groups (genera) (Lawton, 1932; Wagner, 1954; Barrington *et al.*, 1986; Rasbach *et al.*, 1994). Results from the statistical analysis of guard cell size measurements were significantly different between the putative diploid and tetraploid parents, and support a hypothesis of hybridization for the plant in question. We further conclude that the plants in question results from hybridization between *T. kunthii* and *T. ovata* var *lindheimerii*.

Acknowledgments

This work is based in part on a thesis presented in partial fulfillment of the requirement for a Master of Science degree at Sam Houston State University by T. E. Willis. We thank Dr. Alan R. Smith for advice in this investigation. Thanks also to Dr. Karölis Bagdonas, Dr. Andrew Dewees, Dr. Tamara Cook, Dr. Jerry Cook, Dr. Brian Sailer, Walter M. Woodward and Dr. Patrick Nolan.

Literature Cited

Barrington, D. S., C. A. Paris and T. A. Ranker. 1986. Systematic inferences from spore and stomate size in the ferns. Amer. Fern J. 76:149–159.

Brunton, D. F. and W. C. Taylor. 1990. *Isoetes* ×*brittonii* hyb. nov. (Isoetaceae): a naturally occurring hybrid (*I. engelmannii* × *I. riparia*) in the eastern United States. Amer. Fern J. 80:82–89.

Diggs, G. M. Jr., B. L. Lipscomb and R. J. O'Kennon. 1999. *Shinners & Mahler's Illustrated Flora of North Central Texas*. Botanical Research Institute of Texas, Fort Worth.

Hoshizaki, B. J. 2001. Have I found a hybrid? Fiddlehead Forum 28:11.

Klekowski, E. J., Jr. 1969. Reproductive biology of the Pteridophyta. III. A study of the Blechnaceae. Bot. J. Linn. Soc. 62:361–377.

Knobloch, I. W. 1976. Pteridophyte hybrids. Publ. Mus. Michigan State Univ., Biol. Ser. 5:273–352.

Lawton, E. 1932. Regeneration and induced polyploidy in ferns. Amer. J. Bot. 19:303–334.

Mickel, J. T., W. H. Wagner and K. L. Chen. 1966. Chromosome observations on the ferns of Mexico. Caryologia 19:95–102.

Nester, J. E. and M. D. Schedlbauer. 1981. Gametophyte development in *Anemia mexicana* Klotzsch. Bot. Gaz. 142:242–250.

Rasbach, H., T. Reichstein and R. L. L. Viane. 1994. *Aspleniun chihuahuense* (Aspleniaceae, Pteridophyta), an allohexaploid species and the description of a simplified hybridization technique. Amer. Fern J. 84:11–40.

Rieseberg, L. H. and N. C. Ellstrand. 1993. What can molecular and morphological markers tell us about plant hybridization? Critical reviews in plant sciences.12:213–241. CRC Press, Inc., Boca Raton.

Rieseberg, L. H. 1995. The role of hybridization in evolution: Old wine in new skins. Amer. J. Bot. 82:944–953.

Schedlbauer, M. D. 1976. Specificity of the antheridogen from *Ceratopteris thalictroides* (L.)Brongn. Pl. Physiol. 57:666–669.

Smith, A. R. 1971. Systematics of the neotropical species of *Thelypteris* section *Cyclorsorus*. Univ. Calif. Publ. Bot. 59:1–143.

Smith, A. R. 1993. Thelypteridaceae. Pp. 206–222 in Flora of North America Editorial Committee, eds 1993. *Flora of North America, North of Mexico. Vol. 2. Pteridophytes and Gymnosperms*. Oxford University Press, New York.

Wagner, W. H., Jr. 1954. Reticulate evolution in Appalachian *Aspleniums*. Evolution 8:103–118.

Wagner, W. H., Jr. 1969. The role and taxonomic treatment of hybrids. BioScience 19:785–789.

Wagner, W. H., Jr., F. S. Wagner and W. C. Taylor. 1986. Detecting abortive spores in herbarium specimens of sterile hybrids. Amer. Fern J. 76:129–140.

American Fern Journal 96(4):134–138 (2006)

Shorter Notes

Chemotaxonomic survey of flavonoids from *Sphaerostephanos* (Thelypteridaceae) of Peninsular Malaysia.—*Sphaerostephanos* (Thelypteridaceae) is characterized by reduced basal pinnae, sessile non-resinous spherical yellow glands on the upper leaf surface, and a base chromosome number of x=36 (Holttum, R. E., *Flora Malesian. Series II. Pteridophyta. Ferns and Fern Allies. Vol I. pt. 5, Thelypteridaceae*. M. Nijhoff, The Hague. 1982). Little is known about the flavonoid chemistry of Peninsular Malaysia *Sphaerostephanos* species. In this study of the flavonoid distribution of pinnae, we contribute valuable information on inter-specific relationships. Leaves from freshly dried plant material collected from various habitats in Peninsular Malaysia were analysed. Voucher specimens of the ferns (collector number: *UKY 232, UKY 288, UKY 289, UKY 293, UKY 296, UKY 297, UKY 319, UKY 312, UKY 323, UKY 326, UKY 333, UKY 335, NMJ 1, NMJ 2, NMJ 7, NMJ 10, NMJ 11, NMJ 13, NMJ 15, NMJ 19*) have been deposited in the herbarium of the Department of Biology of the Universiti Putra Malaysia, Serdang, Selangor. Standard chromatographic procedures (Harborne, J. B. 1967, *Comparative Biochemistry of the Flavonoids*, Academic Press, London; Harborne, J. B. 1984, *Phytochemical methods*, Chapman and Hall, London; Markham, K. R. 1982, *Techniques of flavonoid Identification*, Academic Press, London) were used for examining flavonoids present in direct and acid hydrolysed leaf extracts; the common aglycones were identified by means of R_f values and color reaction in UV light when compared with standard markers. In acid-hydrolyzed extracts, the flavones were recognized by their distinct, dark yellow spots on paper chromatograms in UV light. When fumed with ammonia vapor they became bright yellow. The flavonols appeared yellow in UV light before and after fuming with ammonia. For complete identification of flavonoid glycosides, samples were separated in one-dimensional chromatograms of direct extracts and then the pure flavonoids were identified by UV spectral analysis using standard procedures of Mabry and coworkers (*The Systematic Identification of the Flavonoids*, Springer-Verlag, New York, 1970). In addition to spectral techniques, flavonoids were identified by PC (Whatman No. 1) co-chromatography of the glycosides and products of enzyme and acid hydrolyses in *n*-butanol-acetic acid-water (BAW, 4:1:5) and 50% glacial acetic acid (50%HOAc). The aglycones were identified by TLC (Merck) co-chromatography in BAW, forestal (concentrated hydrochloric acid-acetic acid-water, 3:30:10) and 30%HOAc, whereas the sugars were identified by PC co-chromatography in BAW, *n*-butanol-ethanol-water (BEW, 4:1:2.2) and toluene-η-butanol-pyridine-water (TBPW, 5:1:3:3).

Twenty populations representing nine species were examined for intraspecific flavonoid variation, but no significant qualitative population level variation was observed in the species studied. Six of the nine *Sphaeroste-*

phanos species examined contain quercetin (Qu) and two of these six also possess kaempferol (Km). Samples of *S. pterocarpus*, *S. norisii* and *S. peltochlamys* do not contain flavonols, but apigenin was found in *S. hendersonii* and *S. heterocarpus*. Isorhamnetin was detected in *S. penniger*, and as far as the authors are aware this is the first report of the presence of isorhamnetin in the Thelypteridaceae.

Ten flavonoids were isolated and purified in this investigation. These compounds were glycosides of flavones and flavonols (Table 1). Flavonol *O*-glycosides appear to be common components of *Sphaerostephanos* (found in 66.6% of the species studied). Thus, quercetin 3-*O*-glucoside, quercetin 3-*O*-galactoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rhamnoside and kaempferol 3-*O*-galactoside were respectively found in 22%, 33%, 22%, 44% and 22% of the species studied. Isorhamnetin 3-*O*-glucoside was detected only in *S. heterocarpus*. Flavone *C*-glycosides and flavone *O*-glycoside seem to have a restricted distribution in this genus and were found in only 22% and 11% of the *Sphaerostephanos* species investigated. Schaftoside and isoschaftoside were found in *S. polycarpus* and apigenin 7-*O*-glucoside was detected in *S. heterocarpus*. In addition, the presence of isorhamnetin 3-*O*-glucoside in *S. hendersonii* and apigenin 7-*O*-glucoside in *S. heterocarpus* is also now reported for the first time in the Thelypteridaceae.

Despite the small sample size, species of *Sphaerostephanos* appear to be divisible into two groups based on the presence of flavonoid glycosides and aglycones (Table 2). However, a more comprehensive survey of this genus is required in order to establish this difference conclusively. Species of Group A contain both flavonoid glycosides and aglycones, whereas species of Group B, completely lack flavonoids. The species of Group A do vary in their glycosides such that it is possible to distinguish some of them by their flavonoid patterns. Two species of *Sphaerostephanos*, *S. polycarpus* and *S. unitus* contain kaempferol, quercetin and kaempferol 3-*O*-rhamnoside. *Sphaerostephanos heterocarpus* accumulates apigenin 7-*O*-glucoside, kaempferol 3-*O*-glucoside and isorhamnetin 3-*O*-glucoside.

Although *S. polycarpus* and *S. unitus* both produce quercetin 3-*O*-glucoside, they can be chemically distinguished from one another because *S. polycarpus* accumulates schaftoside and isoschaftoside whereas *S. unitus* accumulates quercetin 3-*O*-galactoside and kaempferol 3-*O*-galactoside. The last two species, *S. penniger* and *S. larutensis*, have quercetin and quercetin 3-*O*-galactoside, but *S. penniger* produces isorhamnetin as well.

In conclusion, the present work establishes that glycosides of kaempferol are the major flavonoids of *Sphaerostephanos*, being present in 44% of the species examined. Moreover, *Sphaerostephanos* can be subdivided into two groups based on the presence of flavonoids in frond extracts. How far this or any other chemical data can be used to assess the validity of the many competing taxonomic arrangements of the species within *Sphaerostephanos* can only be determined after many more species from other geographical areas have been examined.

TABLE 1. Identification of flavonoid glycosides from the pinnae of *Sphaerostephanos* sp.

Flavonoid glycosides	Color in UV/UV + NH_3	BAW	H_2O	15% HOAc	PhOH	UV spectrum/nm in			
Quercitin 3-glucoside	dark/yellow	67	27	54	49	425. 357. 256	267	379	404
Schaftoside	dark/yellow	72	30	58	52	353, 288	–	–	–
Isoschaftoside	dark/yellow	72	29	59	87	350, 292	–	–	–
Km 3-rhamnoside	dark/yellow	86	28	51	72	352, 267	273	355	392
Quercetin 3-glucoside	dark/yellow	72	15	39	59	359, 267	272	379	388
Kaempferol 3-glucoside	dark/yellow	79	19	41	78	350, 295sh, 266	269	350	390
Kaempferol 3-galactoside	dark/yellow	77	13	43	73	350, 267	267	350	385
Kaempferol 3-rutinoside	dark/yellow	65	31	61	67	350, 268	270	360	402
Apigenin 7-glucoside	dark/yellow	68	10	30	76	336, 265	265	345	381
Isorhamnetin 3-glucoside	dark/yellow	67	24	43	49	350, 266	273	350	385

Key: sh. = shoulder; BAW = η-butanol:acetic acid:water (4:1:5).

TABLE 2. The distribution of flavonoids in the pinnae of *Sphaerostephanos* sp.

Taxon and collector	Flavone *C*-glycosides			Flavones and Flavonols				Flavonol *O*-glycosides					
	SCH	ISCH	Ap 7-glu	Km	Qu	AP	Isorham	Qu 3-glu	Qu 3-gal	Isorham 3-glu	Km 3-glu	Km3-gal	Km 3-gal
Group A:													
S. heterocarpus (Bl.) Holtt.	−	−	+	−	+	+	−	−	−	+	+	+	+
S. hendersonii Holtt.	−	−	+	−	+	+	−	−	−	+	+	+	
S. polycarpus (Bl.) Copel.	+	+	−	+	+	−	−	+	−	−		+	
S. unitus (L.) Holtt.	−	−	−	+	+	−	−	+	+		−	+	+
S. penniger (Hk.) Holtt.	−	−	−	−	+	−	+	−	+			−	−
S. larutensis (Bedd.) C. Chr.	−	−	−	−	+	−	−	−	+	−	−	−	−
Group B:													
S. norisii (Rosenst.) Holtt.	−	−	−	−	−	−	−	−	−	−	−	−	−
S. peltochlamys (C. Chr.) Holtt.	−		−	−	−	−	−	−	−	−	−		
S. pterocarpus (v.A.v.R.) Holtt.			−	−	−	−	−	−	−	−	−	−	−

Key: SCH = schaftoside; ISCH = isoschaftoside; Ap 7-glu = apigenin 7-glucoside; Km = kaempferol; Qu = quercetin; Isorham = isorhamnoside; Qu 3-glu = quercetin 3-glucoside; Qu 3-gal = quercetin 3-galactoside; Isorham 3-glu = isorhamnetin 3-glucoside; Km 3-glu = kaempferol 3-glucoside; Km 3-rha = kaempferol 3-rhamnoside; Km 3-gal = kamepferol 3-galactoside.

The authors thank Universiti Putra Malaysia for financial support.—UMIKALSOM YUSUF, Department of Biology, NANCY MARTINA JACOB, Department of Biology, MOHD ASPOLLAH SUKARI, Department of Chemistry, Universiti Putra Malaysia, Serdang, 43400, and KHAIRUDDIN ITAM, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.

American Fern Journal 96(4):139 (2006)

Referees for 2006

All papers submitted to the journal are peer reviewed. Members of the editorial board and the Society, as well as additional scientists in cognate areas, do these reviews on a voluntary basis. It is their work that contributes to the high quality of articles in the American Fern Journal and to its continued success. The American Fern Society and I extend our thanks to the following reviewers for their assistance, diligence, and patience in the year 2006.

Michael Barker
Gerald Gastony
Gary Greer
Christopher Haufler
Robbin Moran
Pieter Pelser
Tom Ranker
Mick Richardson
Randy Small
Alan Smith
Michael Sundue
Michael Vincent
Tom Wieboldt

STATEMENT OF OWNERSHIP, MANAGEMENT, AND CIRCULATION

Publication title and number: *American Fern Journal* (0002–8444). Date of filing: September 5, 2005. Frequency of issue: quarterly. Annual subscription price 2005: $35.00. Office of Publisher: c/o Missouri Botanical Garden, P.O. Box 299, St. Louis, MO 63166–0299. Editor: R. James Hickey. The *American Fern Journal* is wholly owned by the American Fern Society, Inc., with no bond holders. The purpose, function, and nonprofit status of the Society and its tax exempt status for Federal income tax purposes remains the same as in past years. The average press run for Volume 95 is 1062, of which (for the issue appearing immediately prior to the filing date) 903 copies were mailed as paid circulation and 60 copies were mailed as free distribution, leaving 197 copies for office use and back-issues sales. I certify that these statements are correct and complete. George Yatskievych, Membership Secretary of AFS.

Index to Volume 96

A New Species of *Microlepia* (Dennstaedtiaceeae) from Mt. Micangshan, China, 80
Acrostichum danaeifolium 28
Adiantum 48, 65, 95, 101; *caudatum* 4; *malesianum* 4; *pedatum* 101; *philippense* 4; *tenerum* 4
Agdestis clematidea 23
Aglaomorpha acuminata 9; *heraclea* 9
Alsophila bryophila 104, 108, 109, 110; *erinaceae* 104, 108–110; *polystichoides* 104, 108–110; *salvinii* 28, 108, 109, 110; *setosa*, 103, 104, 107, 108, 109
Amphineuron immersum 14; *terminans* 15
Anemia 48, 96, 123; *adiantifolia* 112
Angiopteris 48; *evecta* 64; *lygodiifolia* 64; *palmiformis* 8; *pruinosa* 8
Antrophyum parvulum 17; *reticulatum* 16; *sessilifolium* 16; *williamsii* 1, 16–18
Araucaria 104
Archangeopteris henryi 64
Aslplenium 17, 48, 65, 93, 96–98, 102; *adiantumn-nigrum* 65; *affine* 4; *caudatum* 4; *cymbifolium* 4; *epiphyticum* 4; *kaulfussii* 65; *lobulatum* 4; *musifolium* 4; *nidus* 4; *pellucidum* 4; *persicifolium* 5; *phyllitidis* 5; *polyodon* 5; *scandens* 5, 17; *scolopendrium* 101; *subnormale* 5; *tenerum* 5; *unilaterale* 5; *vittaeforme* 5; *viviparum* 65
Athyriopsis 97, 98; *longipes* 96, 98, 99
Auld, J. R. (see J. E. Watkins, Jr.)
Austobaileya 48
Azolla 48, 100; *pinnata* 5
Baccaris bigelovii 63
Barcelona, J. F., N. E. Dolotina, G. S. Madroñero, W. G. Granert and D. D. Sopot. The Ferns and Fern Allies of the Karst Forests of Bohol Island, Philippines, 1.
Belvisia mucronata 9
Bicksler, A. J. (see J. E. Watkins, Jr.)
Blechnum 48, 96; *auriculatum* 75; *australe* f. *mucronato-dentatum* 75; *australe* subsp. *auriculatum* 75–77; *laevigatum* 75; *ludificans* 75–79; *orientale* 5
Blechnum ludificans Herter, An Overlooked Fern from South America, 75
Bolbitis angustipinna 115; *bernoullii* 22, 115–125; *cladorrhizans* 115; *costata* 115; *crispatula* 123; *heteroclita* 7, 115, 123; *portoricensis* 115–125; *presliana* 123; *repanda* 115; *semicordata* 115, 123; *subcrenata* 115; 116, 123; *quoyana* 115; *umbrosa* 115–125
Bommeria 95
Botrychium 48, 96, 101
Bradley, K. A. *Thelypteris sancta* (L.) Ching, New for Florida and the Continental United States, 112
Brosmium alicastrum 22
Bursera simaruba 22
Calochlaena 46, 48
Cedrela odorata 22
Cephalomanes atrovirens 7; *brevipes* 7; *humile* 7
Ceratopteris 48; *thalictroides* 9
Ceterach 65
Chang, H.-M. (see W. Sinohara)
Characterization Of A *Thelypteris* Hybrid From Walker County, Texas, 127
Cheilanthes 96; *tomentosa* 102
Cheiropleuria 48
Chemotaxonomic Survey of Flavonoids from *Sphaerostephanos* (Thelypteridaceae) of Peninsular Malaysia, 134
Chingia feros 15
Chloranthus 48
Christella parasitica 15
Cibotium 46; *glaucum* 104, *spp.* 104; *taiwanense* 28
Coccoloba barbadensis 22
Conant, D. S. (see P. Korall)
Coniogramme 48
Ctenitis 1, 31; *boholensis* 1, 3, 13, 18; *humilis* 1, 3, 13, 18; *pallens* 13; *silvatica* 13; sp. 13
Culcita 46
Cyathea 48; *arborea* 104, 109; *caracasana* 104, 110; *contaminans* 5; *delgadii* 104; *hornei* 109; nigripes, 104; *pinnula* 104; *pubescens* 104, 109; sp. 5; *trichiata* 104
Cycas 48
Cyclopeltis presliana 13
Cyclosorus boholensis 1; *glaber* 1, 17
Cystodiumi 45, 46, 48, 50, 51; *sorbifolium* 45, 46, 47
Cystopteris 65, 102; *fragilis* 65
Danaea 48; *wendlandii* 28

Davallia denticulate var. *denticulate* 6; *divaricata* 6; *falcinella* 6, 19; *pectinata* 6; *repens* 6; *solida* 6; *trichomanioides* var. *lorrainii* 6
Davallodes hirsutum 6
Dendrolycopodium 101
Dennstaedtia 48, 50, 51
Deparia 97, 98, 100; ***longipes comb. nov.*** 96, 98, **99**; *petersenii* 97; *prolifera* 97
Deparia longipes (Woodsiaceae) Native to Taiwan, 96
Dicksonia 45, 46, 48, 50
Dicranopteris 48; *linearis* var. *subspeciosa* 6; *linearis* var.? 7
Diphasiastrum 101
Diplazium cf. crenatoserratum 16; *esculentum* 16; *maximum* 16; *nipponicum* 95; *pallidum* 16; *petiolare* 1, 16, 17; *polypodioides* 16; *vestitum* 16
Diplopterygium 48
Dipteris 48
Dolotina, N. E. (see J. F. Barcelona)
Drynaria 19; *descensa* 9, 17, 18; *quercifolia* 9; *rigidula* 9; *sparsisora* 9
Drynaria-type 115, 121
Dryoathyrium 97
Dryopteris 93–96, 100, 102; sect.*Dryopteris* 93; sect. *Erythrovariae* 93; subg. *Ctenitis* 31; *adenopteris* 36; *arguta* 96; *championii* 95; *erythrosora* 93, 95; *gymnophila* 95; *intermedia* 93; *filix-mas* 93, 96; *marginalis* 93, 102; *setigera* 96; *subincisa* 31; *uniformis* 93; *villarii* 93, 96; *villosula* 43; *watanabei* 93; *yungense* 36
Egenolfia 123
Elaphoglossum 123; subg. *Lepidoglossum* 85; *paleaceum* 83–92
Enterolobium cyclocarpum 22
Equisetum 48, 101; *ramosissimum* ssp. *debile* 6
Fern Laminar Scales Protect Against Photoinhibition from Excess Light, 83
Ficus 2; *aurea* 112; *cotinifolia* 22; *obtusifolia* 22
Gametophyte Morphology in Three Mexican Species of *Bolbitis* (Lomariopsidaceae), 115
Gametophytes of Four Tropical, Terrestrial *Huperzia* Species (Lycopodiaceae), 54
Gecarcinus lateralis 27
Ginkgo 48
Giudice, G. E. (see E. R. de la Sota)
Gleichenella 48
Gleichenia 48
Gmelina arborea 2
Gnetum 48
Goniophlebium subauriculatum 9
Granert, W. G. (see J. F. Barcelona)
Growth Rates and Age Estimates of *Alsophila setosa* Kaulf. in Southern Brazil, 103
Guo, X.-S. and B. Li. A New Species of *Microlepia* (Dennstaedtiaceeae) from Mt. Micangshan, China, 80
Gymnopteris 95
Helenium hoopsei 94
Hemionitis 95
Heterogonium aspidioides 13; *pinnatum* 13
Hickey, R. J. Review – Ferns of Northeastern and Central North America, 2nd ed., 100
Hippochaete 48
Hsu, T.-W. W. (see W. Sinohara)
Huperzia 47, 48; *crassa* 54, 57–60; *crassa* var. *gelida* 54; *cumingii* 54, 57–60; *hypogaea* 54, 57–60; *lucidula* 59, 60; *saururus* 54, 57–60; *selago* 60
Hymenophyllopsis 48
Hymenophyllum 48, 96; *polyanthos* 7; *serrulatum* 7
Hypolepis nigrescens 22
Imperato, F. Kaempferol 3-*O*-(acetylrutinoside), A New Flavonoid and Two New Fern Constituents, Quercitin 3-*O*-(acetylglucoside) and 3-*O*-(acetylrutinoside) from *Dryotperis villarii*, 93
Imperato, F. The New Flavone Ester Apigenin 7-*O*-*p*-hydroxybenzoate and Three Di-*C*-Glycosylflavones from *Pteris vittata*, 62
Isoetes 47, 48, 100, 101; *coreana* 67; *echinospora* 71; *hypsophila* 67; *orientalis* 67; *sinensis* 67–74; *sinensis* var. *coreana* 67, 73; *storkii* 72; *taiwanensis* 67; *yunguiensis* 67
Itam, K. (see U. Yusuf)
Jacob, N. M. (see U. Yusuf)
Jacquinia macrocarpa 22
Kaempferol 3-*O*-(acetylrutinoside), A New Flavonoid and Two New Fern Constituents, Quercitin 3-*O*-(acetylglucoside) and 3-*O*-(acetylrutinoside) from *Dryotperis villarii*, 93
Kaiser, K. (see J. E. Watkins, Jr.)
Kawahara, A. Y (see J. E. Watkins, Jr.)
Kessler, M. and A. R. Smith. *Megalastrum* (Dryopteridaceae – Pteridophyta) in Bolivia, with Descriptions of Six New Species, 31

KORALL, P., D. S. CONANT, H. SCHNEIDER, K UEDA, H. NISHIDA and K. M. PRYER. On the Phylogenetic Position of *Cystodium*: It's Not a Tree Fern – It's a Polypod!, 45
Leaf Phenology of the Climbing Fern *Lygodium venustum* in a Semideciduous Lowland Forest on the Gulf of Mexico, 21
Lecanopteris sinuosa 9
LEIGHT, S. A. (see J. E. WATKINS, JR.)
Lemmaphyllum accedens 9
Lepisorus longifolius 9, 17
Leptochilus macrophyllus var. *fluviatilis* 10; *macrophyllus* var. *macrophyllus* 10
Leptopteris 48
LI, B. (see X.-S. GUO)
Libocedrus bidwillii 63
Lindsaea ensifolia var. *ensifolia* 7, 18; *repens* var. *pectinata* forma *angusta* 7
LIU, H., Q. F. WANG AND W. C. TAYLOR. Morphological and Anatomical Variation in Sporophylls of *Isoetes sinensis* Palmer (Isoetaceae), an Endangered Quillwort in China, 67
Lomagramma copelandii 7; *lineata* 8; *merrillii* 1, 7, 17, 18; *pteroides* 8
Lomariopsis recurvata 22
Lonchitis 45, 48, 50
Lophosoria 48
Loxogramme avenia 8; *conferta* 8
Loxoma 48; *cunninghamii* 95
Loxsomopsis 48; *costaricensis* 95
LUNA, M. L. (see E. R. DE LA SOTA)
Lunathyrium 97
Lycopodiella 100, 101
Lycopodium 101; *cernuum* 8; *lucidulum* 59
Lygodium 48, 96; *auriculatum* 12; *circinnatum* 12; *flexuosum* 12; *heterodoxum* 22; *japonicum* 12, 22, 27, 28; *microphyllum* 21, 27; *venustum* 21–29; *volubile* 22
Macrothelypteris torresiana 15
MADROÑERO, G. S. (see J. F. BARCELONA)
Marattia 48, 96
Marsiea 48, 100
Matonia 48
Megalastrum 31; *adenopteris* 32, 36; ***alticola spec. nov.*** 31, 32, 34, **36–37**; ***aureisquama spec. nov.*** 31, 32, 34, 36, **37–38**; *biseriale* 32, 33, 35, 36; ***bolivianum spec. nov.*** 31, **33–35**; ***ciliatum spec. nov.*** 31, 32, **38–40**, 40; *connexum* 42 *hirsutosetosum* 35 *honestum* 32, 33, 35; ***marginatum spec. nov.*** 31, 32, 38, **40**; *pulverulentum* 31, 32, 40, 40; *pulverulentum* var. *heydei* 41; ***rupicola spec. nov.*** 31, 32, 36, 38, 40, **41–42**; *subincisum* 31, 32, 36–38, 41–43; *vastatum* 32, 43; *villosulum* 32, 43, 44; *yungense* 32, 33, 36
Megalastrum (Dryopteridaceae – Pteridophyta) in Bolivia, with Descriptions of Six New Species, 31
MEHLTRETER, KLAUS. Leaf Phenology of the Climbing Fern *Lygodium venustum* in a Semideciduous Lowland Forest on the Gulf of Mexico, 21
MENDOZA-RUIZ, A. (see B.PÉREZ-GARCÍA)
Metaxya 48, 49
Microlepia 48; ***micanghanensis spec. nov.*** **80–81**; *sino-strigosa* 81; *speluncae* 6;
Microsorum 17, 19; *commutatum* 10; *heterocarpum* 10; *longissimum* 10; *membranifolium* 10; *monstrosum* 10; *punctatum* 10; *rubidium* 10; *samarense* 10; *scolopendria* 10; *zippelii* 10
Mohria 96
Monachosorum 48
MOORE, S.-J. (see W. SINOHARA)
MORAN, R. C. Review – Flora de la República de Cuba: Series A. Plantas Vasculares. Fascículo 11., 66
Morphological and Anatomical Variation in Sporophylls of *Isoetes sinensis* Palmer (Isoetaceae), an Endangered Quillwort in China, 67
MURAKAMI, N. (see W. SINOHARA)
Nectandra salicifolia 22
Nephrolepis biserrata 8; *falcata* 8; *multiflora* 8
NESTER-HUDSON J. E. (see T. E. WILLIS)
NISHIDA, H. (see P. KORALL)
Notholaena 63, 96
Oleandra 1; *cf. benguetensis* 8
On the Phylogenetic Position of *Cystodium*: It's Not a Tree Fern – It's a Polypod!, 45
Onoclea 100
Ophioglossum 48, 100, 101; *petiolatum* 9
Osmunda 48, 101
Paesia anfractuosa 95
Paullinia tomentosa 23
PÉREZ-GARCÍA, B. and A. MENDOZA-RUIZ. Gametophyte Morphology in Three Mexican Species of *Bolbitis* (Lomariopsidaceae), 115
Phanerosorus 48
Pilularia 48
Pinus 48; *elliottii* var. *densa* 112
Piper amalago 22

Pityrogramma calomelanos 4
Plagiogyria 48
Platycerium coronarium 10, 17, 19; *grande* 17
Pleocnemia irregularis 13; *presliana* 13
Pleopeltis 100; *polypodioides* 85; *vulgare* 85
Plumeria floribunda 94
Pneumatopteris glabra 1, 15, 17; *laevis* 15; *ligulata* 15; *nitidula* 15
Podocarpus 104
Polypodium biseriale 33, *decumanum* 95; *honestum* 35; *pulverulentum* 40; *subincisum* 42; *vastum* 43; *vulgare* 64
Pronephrium asperum 15; *rhombeum* 15; x*xiphoides* 15
Pryer, K. M. (see P. Korall)
Pseudolycopodiella 100, 101
Psilotum 48; *nudum* 11
Psomiocarpa apiifolia 13
Pteridium 48, 96, 100; *aquilinum* 65, 95; *aquilinum* var. *wrightianum* 6; *caudatum* 6
Pteridrys microtheca 13
Pteris 17, 96; *armata* 11; *ensiformis* 11; *mertensoides* 11; *opaca* 11, 19; *oppositi-pinnata* 11; *pellucida* 11; *philippinensis* 11; *tripartitia* 11; *vittata* 11, 62–65, 95; *whitfordii* 1, 12
Pyrrosia lanceolata 10; *longifolia* 10; *nummularifolia* 11; *piloselloides* 11; *spledens* 11
Quercus virginiana 112
Rafflesia 2
Randia monantha 23
Review – Ferns of Northeastern and Central North America, 2nd ed., 100
Review – Flora de la República de Cuba: Series A. Plantas Vasculares. Fascículo 11., 66
Saccoloma 45, 48, 50
Salvinia 48
Schinus terebinthifolius 112
Schizaea 48,100; *inopinnata* 12
Schmitt, J. L. and P. G. Windisch. Growth Rates and Age Estimates of *Alsophila setosa* Kaulf. in Southern Brazil, 103
Schneider, H. (see P. Korall)
Selaginella 1, 17, 47, 48, 101; *aristata* 12; *cupressina* 12; *delicatula* 12; *engleri* 12; *flagellifera* 12; *involvens* 12; *lepidophylla* 85; *llanosii* 12
Sinohara, W., T.-W. Hsu, S.-J. Moore, H.-M. Chang and N. Murakami. *Deparia longipes* (Woodsiaceae) Native to Taiwan, 96
Smith, A. R. (see M. Kessler)
Sopot, D. D. (see J. F. Barcelona)
Sota, de la, E. R., G. E. Giudice and M. L. Luna. *Blechnum ludificans* Herter, An Overlooked Fern from South America, 75
Sphaeropteris cooperi 104
Sphaeropteris senilis 104, 109
Sphaerostephanos 134–137; *acrostichoides* 1, 15; *hendersonii* 135, 137; *heterocarpus* 15, 135, 137; *larutensis* 135, 137; *norisii* 135, 137; *peltochlamys* 135, 137; *penniger* 135, 137; *polycarpus* 135, 137; *productus* 16; *pterocarpus*, 135, 137; *unitus* 16, 135, 137
Sphenomeris 45, 48, 50; *retusa* 7
Spinulum 101
Sticherus 48
Stromatopteris 48
Strongylodon 2
Sukari, M. A. (see U. Yusuf)
Swietenia macrophylla 2
Taenitis cordata 4, 18
Taylor, W. C. (see H. Liu)
Tectaria 17; *angulata* 14; *athyriosora* 14; *aurita* 14; *calcarea* 14; *crenata* 14; *decurrens* 14; *devexa* 14; *dissecta* 14; *lobbii* var. *allosora* 19; *lobbii* var. *denticulata* 19; lobbii var. *lobbii* 14; *melanocaula* 14; *ramosii* 14; *cf. villosa* 14
Tetraphyllum aculeatum 8
Tetrastigma 2
The Ferns and Fern Allies of the Karst Forests of Bohol Island, Philippines, 1.
The New Flavone Ester Apigenin 7-*O*-*p*-hydroxybenzoate and Three Di-*C*-Glycosylflavones from *Pteris vittata*, 62
Thelypteris 48
Thelypteris 48; subgenus *Amauropelta* 112; subgenus *Amauropelta* 112; subgenus *Cyclosorus* 112; *augescens* 127; *dentata* 112; *kunthii* 112, 127–132; *ovata* var. *lindheimeri* 127–132; *ovata* var. *ovata* 127; *palustris* 95; *patens* 112; *resinifera* 112; *sancta* 112–114; *sevillana* 1, 17
Thelypteris sancta (L.) Ching, New for Florida and the Continental United States, 112
Thylacopteris papillosa 11
Thyrsopteris 46
Tmesipteris 48
Todea 48
Trachypteris 95
Trichomanes 48, 96
Ueda, K. (see P. Korall)
Vitis bourgaeana 23
Vittaria elongata 16

Vittaria-type 115, 117, 121
Wang, Q. F. (see H. Liu)
Watkins, J. E., A. Y. Kawahara, S. A. Leicht, J. R. Auld, A. J. Bicksler and K. Kaiser. Fern Laminar Scales Protect Against Photoinhibition from Excess Light, 83
Whittier, D. P. Gametophytes of Four Tropical, Terrestrial *Huperzia* Species (Lycopodiaceae), 54
Willis, T. E. and J. E. Nester-Hudson. Characterization Of A *Thelypteris* Hybrid From Walker County, Texas, 127
Windisch, P. G. (see J. L.Schmitt)
Yusuf, U., N. M. Jacob, M. A. Sukari, and K. Itam. Chemotaxonomic Survey of Flavonoids from *Sphaerostephanos* (Thelypteridaceae) of Peninsular Malaysia, 134

INFORMATION FOR AUTHORS

Authors are encouraged to submit manuscripts pertinent to pteridology for publication in the *American Fern Journal*. Manuscripts should be sent to the Editor. Acceptance of papers for publication depends on merit as judged by two or more referees. Authors are encouraged to contribute toward publishing costs; however, the payment or non-payment of page charges will affect neither the acceptability of manuscripts nor the date of publication.

Authors should adhere to the following guidelines; manuscripts not so prepared may be returned for revision prior to review. Submit manuscripts in **triplicate** (xerox copies acceptable), including review copies of illustrations and originals of illustrations. After review, submission of final versions of manuscripts on diskette (in PC- or Mac-compatible formats) is strongly encouraged. Use standard 8½ by 11 inch paper of good quality, not "erasable" paper. **Double space manuscripts throughout**, including title, authors' names and addresses, short, informative abstract, text (including heads and keys), literature cited, tables (separate from text), and figure captions (grouped as consecutive paragraphs separate from figures). Arrange parts of manuscript in order just given. Include author's name and page number in upper right corner of every sheet. Provide margins of at least 25 mm all around on typed pages. Do not submit right-justified copy, avoid footnotes, and do not break words at ends of lines. Make table headings and figure captions self-explanatory. Use S.I. (metric) units for all measures (e.g., distance, elevation, weight) unless quoted or cited from another source (e.g., specimen citations). For nomenclatural matter (i.e., synonymy and typification), use one paragraph per basionym (see Regnum Veg. 58:39–40. 1968). Abbreviate titles of serial publications according to *Botanico-Periodicum-Huntianum* (Lawrence et al., 1968, Hunt Botanical Library, Pittsburgh) and its supplement (1991). References cited only as part of nomenclatural matter are not included in literature cited. For shorter notes and reviews, omit the abstract and put all references parenthetically in text. Use *Index Herbariorum* (Regnum Veg. 120:1–693. 1990) for designations of herbaria.

Illustrations should be proportioned to fit page width with caption on the same page. Provide margins of at least 25 mm on all illustrations. For continuous-tone illustrations, design originals for reproduction without reduction or by uniform amount. In composite blocks, abut edges of adjacent photographs. Avoid combining continuous-tone and line-copy in single illustrations or blocks. Coordinate sequence and numbering of figures (and of tables) with order of citation in text. Explain scales and symbols in figures themselves, not in captions. Include a scale and reference to latitude and longitude in each map.

Proofs and reprint order forms are sent to authors by the printer. Authors should send corrected proofs to the editor and reprint orders to the printer. Authors will be assessed charges for extensive alterations made after type has been set.

For other matter of form or style, consult recent issues of *American Fern Journal* and *The Chicago Manual of Style*, 14th ed. (1993, Univ. Chicago Press, Chicago). Occasionally, departure from these guidelines may be justified. Authors are encouraged to consult the editor for assistance with any aspect of manuscript preparation.

Papers longer than 32 printed pages may be sent to the Editor of *Pteridologia* (Memoir Editor, see cover 2).

PTERIDOLOGIA ISSUES IN PRINT

The following issues of *Pteridologia*, the memoir series of the American Fern Society, are available for purchase:

1. Wagner, David H. 1979. Systematics of Polystichum in Western North America North of Mexico. 64 pp. $10.00 postpaid.

2A. Lellinger, David B. 1989. The Ferns and Fern-allies of Costa Rica, Panama, and the Chocó (Part 1: Psilotaceae through Dicksoniaceae). 364 pp. $32.00 postpaid.

3. Lellinger, David B. 2002. A Modern Multilingual Glossary for Taxonomic Pteridology. 263 pp. $28.00 postpaid.

Send your order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

AMERICAN FERN JOURNAL ON MICROFICHE

Volumes 1–61 of the *American Fern Journal* are available as archival quality, silver positive microfiches. Single volumes or the entire run may be purchased. The fiches are easily read with 10x or greater magnification (using a dissecting microscope and transmitted illumination or a fiche reader). Silver negative microfiches of vols. 1–50 are also available. The price is $4.00 per volume or $244.00 per set of 61 volumes, postpaid.

Send your inquiry or order with a check or money order to: American Fern Society, Inc., % Ecology III, 804 Salem Blvd., Berwick, PA, 18603-9801.

FIDDLEHEAD FORUM

The editor of the Bulletin of the American Fern Society welcomes contributions from members and non-members, including miscellaneous notes, offers to exchange or purchase materials, personalia, horticultural notes, and reviews of non-technical books on ferns.

SPORE EXCHANGE

Ms. Denia Mandt, 12616 Ibbetson Ave., Downey, CA 90242-5050, is Director. Spores exchanged and lists of available spores sent on request. http://amerfernsoc.org/sporexy.html

GIFTS AND BEQUESTS

Gifts and bequests to the Society enable it to expand its services to members and to others interested in ferns. Back issues of the Journal and cash or other gifts are always welcomed and are tax-deductible. Inquiries should be addressed to the Secretary.

VISIT THE AMERICAN FERN SOCIETY'S WORLD WIDE WEB HOMEPAGE: http://www.amerfernsoc.org/